# EXHIBIT 1

#### US008273308B2

### (12) United States Patent Handique et al.

### MOVING MICRODROPLETS IN A

MICROFLUIDIC DEVICE

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U.S.C. 154(b) by 328 days.

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#### Related U.S. Application Data

- (63) Continuation of application No. 10/075,371, filed on Feb. 15, 2002, now Pat. No. 7,323,140, which is a continuation-in-part of application No. 10/014,519, filed on Dec. 14, 2001, now Pat. No. 7,192,557, and a continuation-in-part of application No. 09/953,921, filed on Sep. 18, 2001, now Pat. No. 6,575,188, and a continuation-in-part of application No. 09/819,105, filed on Mar. 28, 2001, now Pat. No. 7,010,391.
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- (51) Int. Cl.

  G01N 15/06 (2006.01)

  G01N 33/00 (2006.01)

  G01N 33/48 (2006.01)

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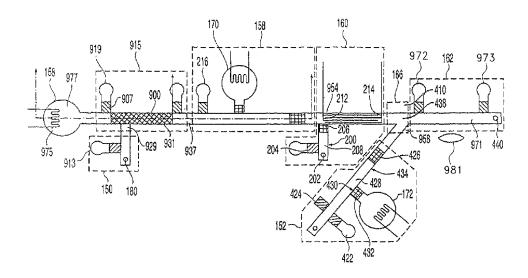
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#### (57) ABSTRACT

This disclosure provides systems, methods, and devices for processing samples on a microfluidic device. One system includes a microfluidic device having an upstream channel, a DNA manipulation zone located downstream from the upstream channel and configured to perform PCR amplification of a sample, a first valve disposed upstream of the DNA manipulation zone, and a second valve disposed downstream of the DNA manipulation zone. The system also includes a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the DNA manipulation zone, and a computer-controlled heat source in thermal contact with the DNA manipulation zone.

#### 25 Claims, 15 Drawing Sheets



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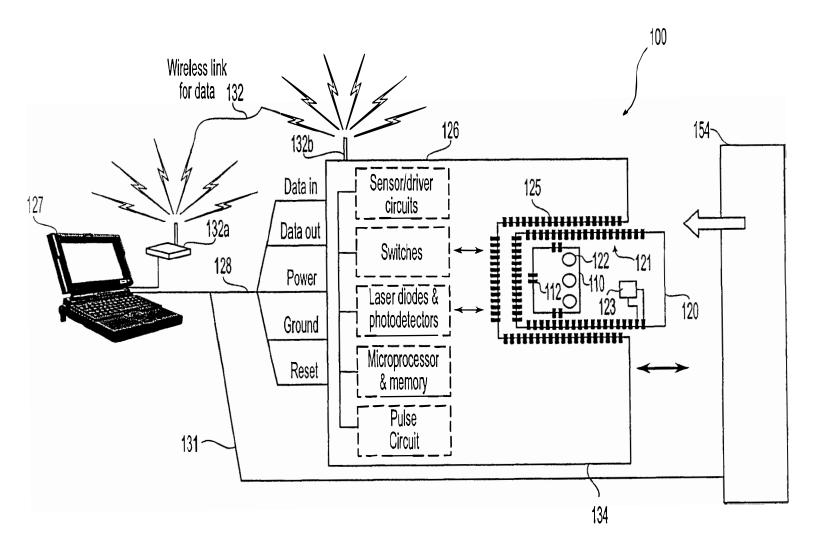
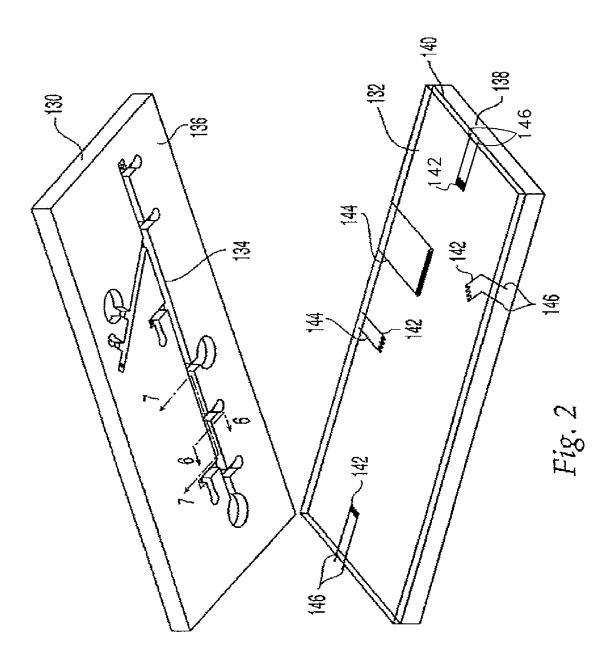
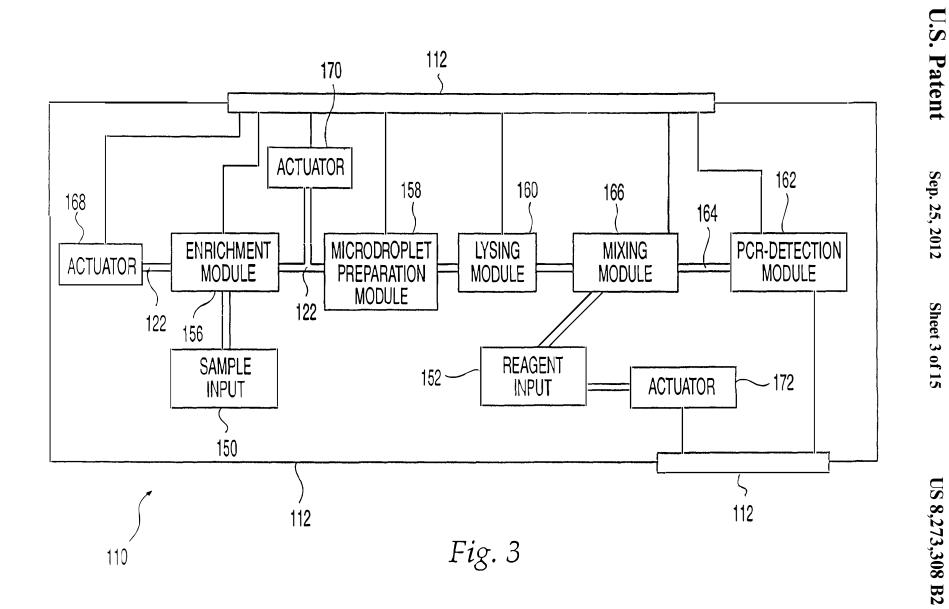


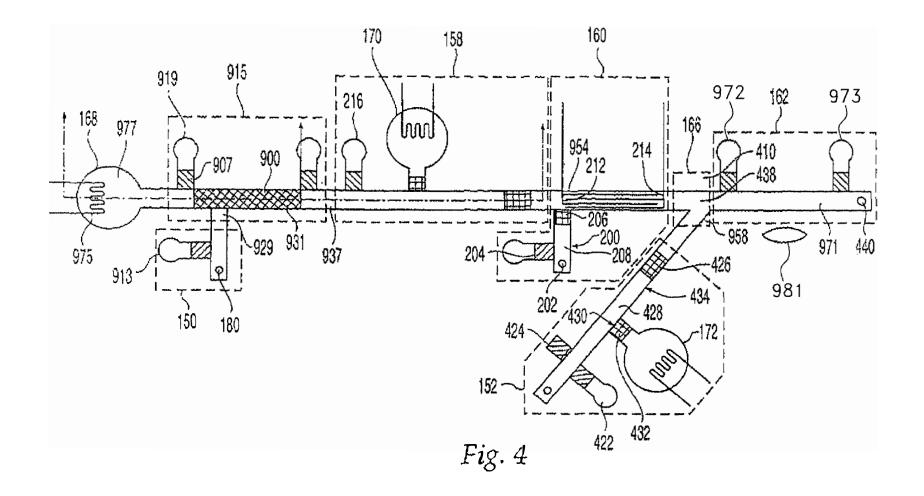
Fig. 1

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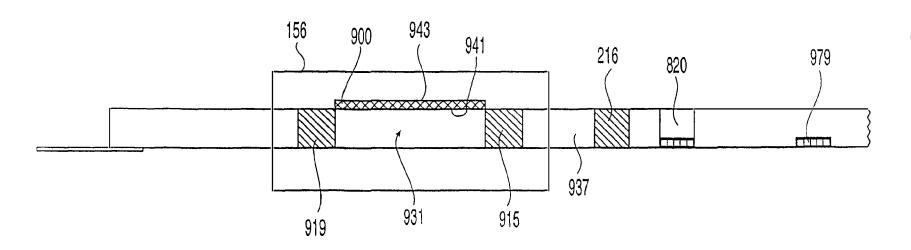


Fig. 5

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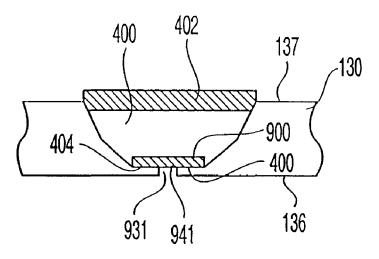


Fig. 6

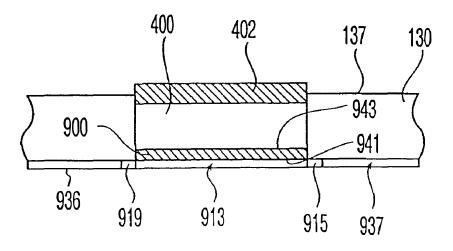


Fig. 7

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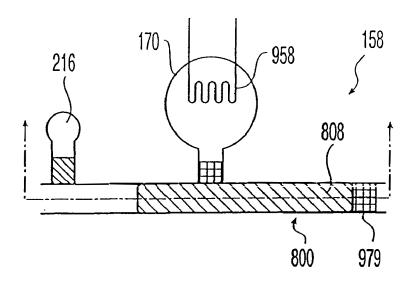


Fig. 8a

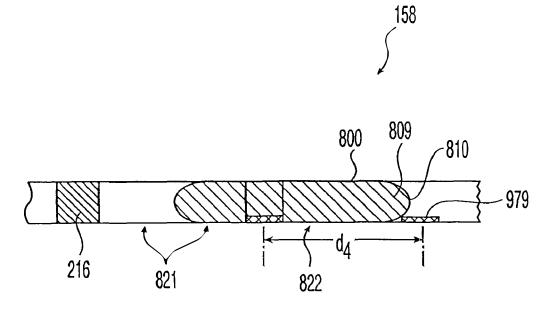


Fig. 8b

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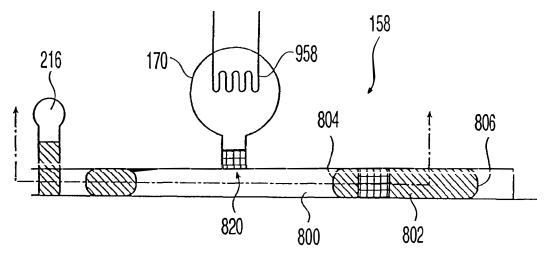


Fig. 9a

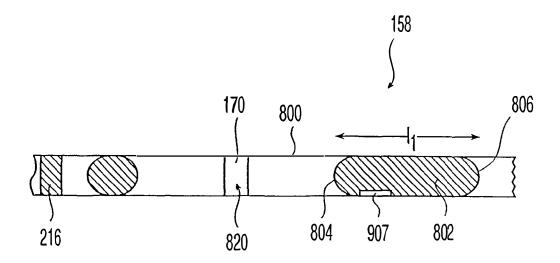
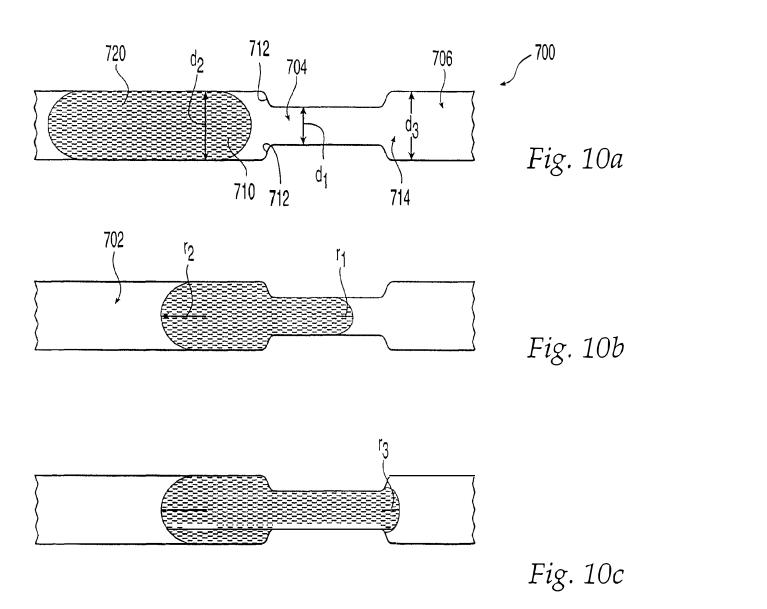
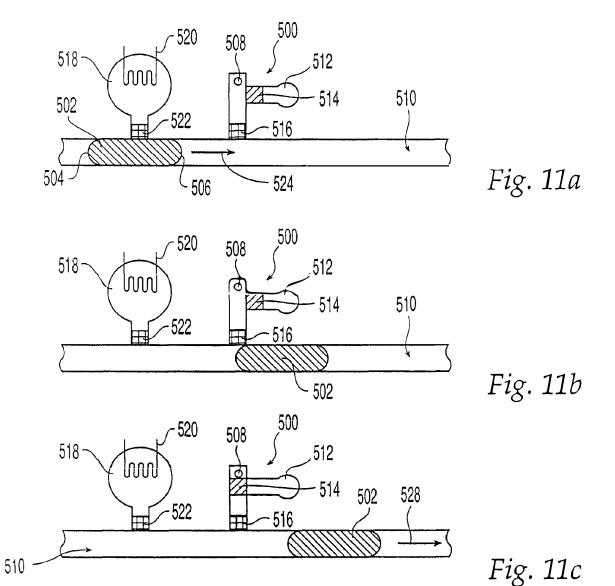


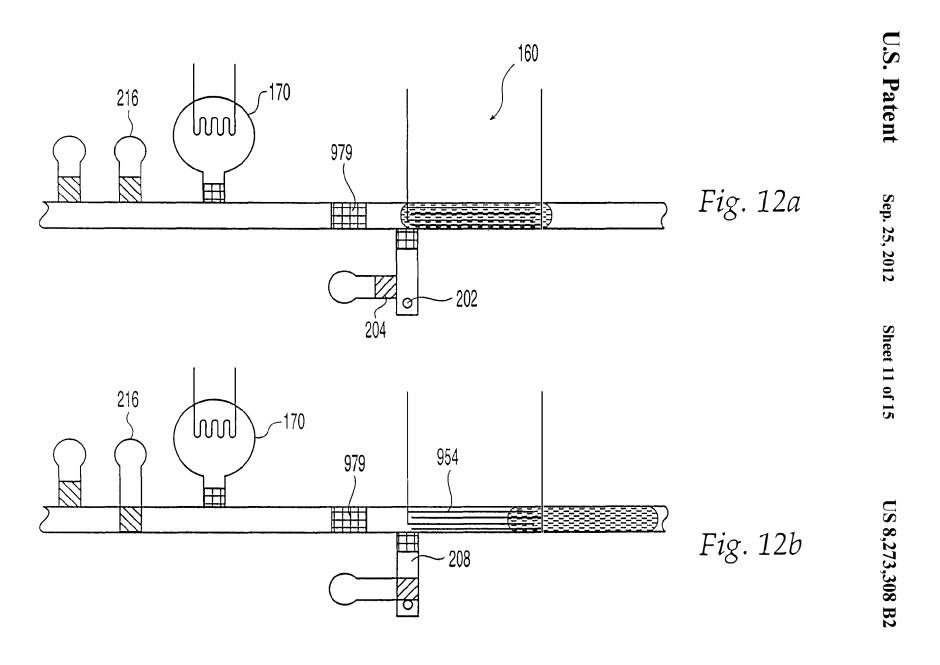
Fig. 9b



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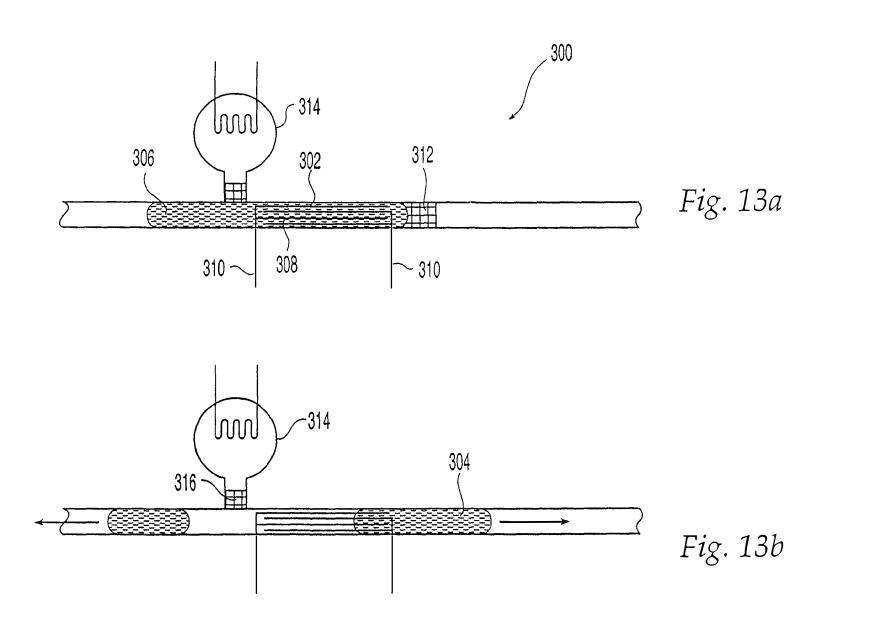
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Fig. 14

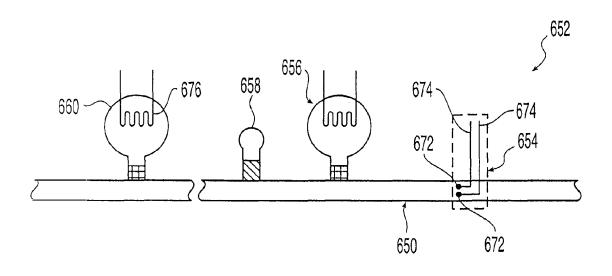


Fig. 15a

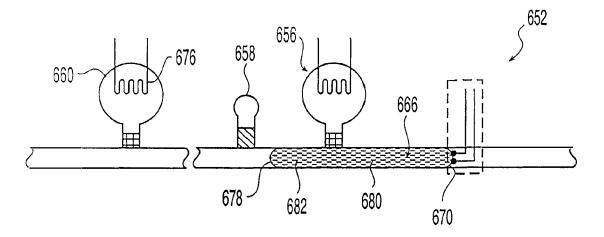


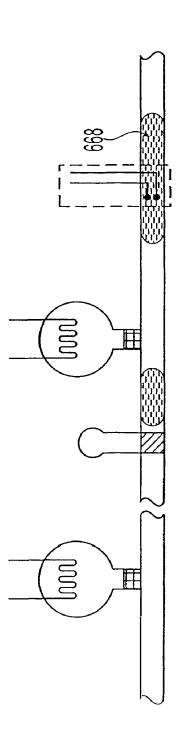
Fig. 15b

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Fig. 15c



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#### MOVING MICRODROPLETS IN A MICROFLUIDIC DEVICE

#### RELATED APPLICATIONS

This application is a continuation application of and claims priority to U.S. application Ser. No. 10/075,371, filed on Feb. 15, 2002, now U.S. Pat. No. 7,323,140 which is a continuation-in-part of application Ser. No. 10/014,519, filed Dec. 14, 2001 now U.S. Pat. No. 7,192,557. This application is also a  $^{10}$ continuation-in-part of application Ser. No. 09/953,921, filed Sep. 18, 2001, now U.S. Pat. No. 6,575,188 and claims priority of provisional Application No. 60/307,638 filed Jul. 26, 2001. This application is also a continuation-in-part of appli-  $_{15}$ cation Ser. No. 09/819,105, filed Mar. 28, 2001 now U.S. Pat. No. 7,010,391. Each of the above-mentioned applications are incorporated herein by reference.

#### FIELD OF THE INVENTION

The present invention relates to methods and systems for processing samples using microfluidic systems. More particularly, the invention relates to moving fluid samples within a microfluidic system.

#### BACKGROUND

Microfluidic devices are typically formed of substrates (made of silicon, glass, ceramic, plastic and/or quartz) which 30 include a network of micro-channels through which fluid flows under the control of a propulsion mechanism. The micro channels typically have at least one dimension which is on the order of nanometers to hundreds of microns.

Microfluidic devices process minute amounts of fluid 35 following drawings, in which: sample to determine the physical and chemical properties of the sample. Microfluidic devices offer several advantages over a traditional macro-scale instrumentation. For example, in general, they require substantially smaller fluid samples, use far less reagent, and process these fluids at substantially 40 microfluidic system of FIG. 1; greater speeds than macro-scale equipment.

Electric fields are used as a propulsion mechanism for some microfluidic devices. In such devices, a high voltage, on the order of kilovolts, is applied across electrodes within the device to thereby generate an electric field in the micro chan- 45 nels. The field imposes a force on ions within the fluid, thereby propelling the ions through the micro channel. The fluid itself may also be propelled by the motion of ions moving within the fluid.

channels. In some devices, a source of pressurized gas, external to the microfluidic device, is connected to the microfluidic device to supply a gas pressure, which propels the fluid. Gas pressure may also be generated by a heated chamber within channel.

#### SUMMARY OF THE INVENTION

In general, the invention relates to a system and method for 60 moving samples, such as fluids, within a microfluidic system. In one aspect, the invention relates to the use of a plurality of gas actuators for applying pressure at different locations within the microfluidic system to thereby supply force for moving samples. For example, in one embodiment, a first gas 65 actuator provides a gas pressure sufficient to move a first sample from a first location to a second location of the microf2

luidic device. A second gas actuator provides a gas pressure to move another sample from a third location to a fourth location of the microfluidic device.

In another example, a plurality of gas actuators cooperate to move the same fluid sample. A first gas actuator provides a gas pressure sufficient to move the microdroplet between first and second processing zones of the microfluidic device, and a second gas actuator provides a gas pressure to move the microdroplet to a third processing zone.

In preferred embodiments, the plurality of actuators are integral with a microfluidic network through which the microfluidic samples flow. For example, a plurality of gas actuators can be fabricated in the same substrate which forms the microfluidic network. One such gas actuator is coupled to the network at a first location for providing gas pressure to move a microfluidic sample within the network. Another gas actuator is coupled to the network at a second location for providing gas pressure to further move at least a portion of the microfluidic sample within the network.

In other aspect, the invention relates to the use of valves with the plurality of actuators. For example, in one embodiment, a valve is coupled to a microfluidic network so that, when the valve is closed, it substantially isolates the second gas actuator from the first gas actuator. Such valves can control the direction of the propulsive force of the actuators by preventing the expanding gas from traveling in certain directions, while permitting it to expand in the desired direction. They also extend the range over which an actuator can propel a microdroplet, by preventing the gas from dissipating in certain in areas upstream from the microdroplet.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is described below in reference to the

FIG. 1 shows a microfluidic system according to the invention;

FIG. 2 shows an expanded view of a microfluidic device.

FIG. 3 shows a schematic of a microfluidic device of the

FIG. 4, shows a top view of the microfluidic device of FIG.

FIG. 5 shows a partial cross-sectional view of the microfluidic device of FIG. 4;

FIG. 6 shows a partial cross-sectional view of an upper substrate from the microfluidic device of FIG. 2;

FIG. 7 shows a second partial cross-sectional view of an upper substrate from the microfluidic device of FIG. 2;

FIG. 8a shows a top view of a microdroplet preparation Gas pressure is also used to propel fluid through micro 50 zone of the microfluidic device of FIG. 4 before preparation of a microdroplet;

> FIG. 8b shows cross sectional view of the microdroplet preparation zone of FIG. 8a;

FIG. 9a shows a top view of a microdroplet preparation the microfluidic device itself to propagate fluid within a micro 55 zone of the microfluidic device of FIG. 4 after preparation of a microdroplet;

> FIG. 9b shows a cross sectional side view of the microdroplet preparation zone of FIG. 9a;

FIGS. 10a-10c show cross sectional side views of a capillary assisted fluid barrier of the present invention;

FIGS. 11a-11c show top views of a fluid barrier comprising

FIGS. 12a and 12b show top views of the lysing module of the microfluidic device of FIG. 4, before and after preparation of a lysed sample;

FIGS. 13a and 13b show a second embodiment of a lysing module of the invention;

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FIG. 14 shows a pulsing circuit associated with the lysing module of FIG. 4; and

FIGS. 15a-15c show a second microdroplet preparation module of the invention.

#### DETAILED DESCRIPTION OF A PREFERRED **EMBODIMENT**

The present invention relates to microfluidic systems and methods for processing materials, such as samples and 10 reagents. More specifically, the invention relates to microfluidic systems and methods for moving fluids within a microfluidic system. In the embodiment described below, the fluid includes particles which tend to move with the fluid. The fluid component of the particle-containing fluid is a gas or, preferably, a liquid. The particles of the particle-containing fluid are preferably whole cells, such as bacterial cells or cells of an animal, such as a human. However, they may include intracinvention may be used to process a sample of bacterial cells to determine whether the bacteria are pathogenic.

#### A. System Overview

FIG. 1 depicts a microfluidic system 100 that includes a microfluidic device 110 and corresponding cartridge 120, 25 which receive one or more fluid samples and process the samples under the control of computer 127 and data acquisition and control board (DAQ) 126.

Computer 127 preferably performs high level functions, such as supplying a user interface that allows a user to select desired operations, notifying the DAQ 126 as to the selected operations, and displaying for the user the results of such operations. These operations include, for example, subjecting a sample to process steps within the various process zones of the microfluidic device. The computer 127 may be a portable computer to facilitate transport of the microfluidic system.

Computer 127 is connected to DAQ 126 via connection 128, which provides data I/O, power, ground, reset, and other functional connectivity. Alternatively, a wireless link 132 between the computer 127 and the DAQ 126 may be provided for data and control signal exchange via wireless elements 132(a) and 132(b). Where the data link is a wireless link, for example, the DAQ 126 may have separate power source, such as a battery.

In general, DAQ 126 controls the operation of microfluidic device 110 in accordance with the high level instructions received from computer 127. More specifically, to implement a desired operation requested by computer 127, DAQ 126 supplies the appropriate electrical control signals to cartridge 50 120 via contacts 125.

Cartridge 120 provides electrical and optical connections 121 for electrical and optical signals between the DAQ 126 and the microfluidic substrate 110, thereby allowing DAQ **126** to control the operation of the substrate.

The chip carrier cartridge 120 is shown being inserted into (or removed from) an interface hardware receptacle of the DAQ 126 having electrical and optical contacts 125 standardized to mate with a corresponding contacts 121 of the chip carrier cartridge 120. Most contacts are for electrical signals, 60 while certain ones are for optical signals (IR, visible, UV, etc.) in the case of optically-monitored or optically-excited microfluidic processors. Alternatively (not shown), the entire DAQ 126 may be a single ASIC chip that is incorporated into the Chip Carrier Cartridge 120, wherein contacts 121,125 65 would become conductive pathways on a printed circuit board.

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B. Microfluidic Device

FIG. 2 illustrates the general structure of a preferred type of microfluidic device. The device includes an upper substrate 130, which is bonded to a lower substrate 132 to form a fluid network.

The upper substrate 130 depicted in FIG. 2 is preferably formed of glass and has a microfluidic network 134 in its bottom surface 136. Those skilled in the art will recognize that substrates composed of silicon, glass, ceramic, plastic, and/or quartz are all acceptable in the context of the present invention.

The microfluidic network includes a plurality of zones. The number of zones, as well as the overall topology of the microfluidic network, will depend upon the particular application which the microfluidic device is designed to perform. The zones of the microfluidic device may have any crosssectional shape, such as generally arcuate or generally polygonal. For example, a zone may include channels, chambers or other substantially enclosed spaces. By "substantially ellular material from such cells. For example, a system of the 20 enclosed" it is meant that materials enter or exit the zones only through predetermined pathways. Examples of such pathways include channels, microchannels and the like, which interconnect the various zones. The zones preferably have at least one micro-scale dimension, such as less than about 250 µm or, more preferably, less than about 75 µm.

> The channels and chambers of the microfluidic network are etched in the bottom surface 136 of the upper substrate 130 using known photolithographic techniques. More specifically, transparent templates or masks containing opaque designs are used to photo-define objects on the surface of the substrate. The patterns on the templates are generated with computer-aided-design programs and can delineate structures with line-widths of less than one micron. Once a template is generated, it can be used almost indefinitely to produce identical replicate structures. Consequently, even extremely complex microfluidic networks can be reproduced in mass quantities and at low incremental unit cost. Alternatively, if a plastic material is used, the upper substrate may be formed using injection molding techniques, wherein the micro-channels are formed during the molding process.

> The lower substrate 132 may include a glass base 138 and an oxide layer 140. Within oxide layer 140, resistive heaters 142 and electric leads 144 are formed using photo-lithographic techniques. The leads 144 connect to terminals 146 which are exposed at the edge of the substrate to permit electrical connection to cartridge 120, thereby permitting DAO 126 to control the heaters. More specifically, to activate a heater 142, DAQ 126 applies a voltage across a pair of terminals 146 (via cartridge 120) to supply current through leads 146 and heater 142, thereby heating the resistive heater element 142.

Metal heater elements 142 are positioned so that, when the upper and lower substrates are bonded together, the heaters reside directly beneath certain regions of the fluid network of the upper substrate so as to be able to heat the contents of these regions. The silicon oxide layer 140 prevents the heating elements 142 from directly contacting with material in the microfluidic network.

The oxide layer 140, heating elements 142, and resistive leads 144 are fabricated using well-known photolithographic techniques, such as those used to etch microfluidic network.

FIG. 3 illustrates a top-down view of microfluidic device 110. As shown, the substrate has a sample input module 150 and reagent input module 152 to allow sample and reagent materials, respectively, to be input to device 110. Preferably, input modules 150, 152 are disposed to allow automatic material input using a computer controlled laboratory robot 154.

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The substrate also includes process modules 156, 158, 160, 166 and 162 for processing the sample and reagent materials. Within these process modules, a sample may be subjected to various physical and chemical process steps. For example, enrichment module 156 prepares a fluid sample having a 5 relatively high concentration of cell particles, lysing module 160 releases intracellular material from the cell particles, and mixing module 166 mixes the resultant sample with certain reagents. As another example, an amplification process modof DNA within a sample.

Various modules of microfluidic device 110 are connected, such as by channels 164, to allow materials to be moved from one location to another within the device 110. Actuators 168, 170, 172 associated with the microfluidic device provide a 15 motive force, such as a gas pressure, to move the sample and reagent material along the channels and zones. For example, a first actuator 168 moves material downstream from process module 156 to process module 158. Upon completion of processing within process module 158, a second actuator 170 20 moves material downstream to mixing process module 160. Subsequently, actuator 170 or an additional actuator moves the material to mixing module 166, where the material mixes with a reagent moved by actuator 172. Finally, actuator 172, or another actuator, moves the mixed material to module 162. 25

Because each actuator is preferably responsible for moving materials within only a subset of the modules of device 110, sample materials can be controlled more precisely than if a single actuator were responsible for moving material throughout the entire device. The various functional ele- 30 ments, of microfluidic device 110, including the actuators, are preferably under computer control to allow automatic sample processing and analysis.

#### C. Multiple Actuators

The various actuators of microfluidic device 110 cooperate 35 to move material between different locations of microfluidic device 110. For example, actuator 168 moves material, such as an enriched sample, between an enrichment zone 931 and a microdroplet preparation module 158. Actuator 170 prepares a microdroplet from the enriched sample and, in so 40 doing, moves the microdroplet to a lysing zone 950. Actuator 170 is used to move material from the lysing zone 950 to mixing module 166. It should be noted, however, that another actuator may be disposed intermediate between lysing zone 950 and microdroplet preparation zone to move the lysed 45 sample downstream to the mixing module 166.

Actuators of device 110 may also cooperate in moving two amounts of material simultaneously. For example, as described above, actuator 172 and actuator 170 cooperate to mix reagent and lysed microdroplets. Such cooperative actua- 50 tors can be controlled independently of one another to ensure proper mixing. For example, if one material is known to be more viscous, the motive force moving that material can be increased independently of the motive force moving the other

The multiple actuators and modules of microfluidic device 110 are preferably operatively connectable and isolatable by the valves of microfluidic device. For example, a closed state of either of valves 915, 216 operatively isolates microdroplet preparation module 170 from enrichment module 156. Thus, 60 one or more actuators can be used to move materials between predetermined locations within microfluidic device 110, without perturbing or contacting material present in an operatively isolated module. The ability to operatively connect and isolate desired modules is advantageous in microfluidic 65 devices having many process functions. Further, these valves also control the direction of the propulsive force of the actua6

tors by preventing the expanding gas from traveling in certain directions, while permitting it to expand in the desired direction. This also extends the range over which an actuator can propel a microdroplet, by preventing the gas from dissipating in certain in areas upstream from the microdroplet.

The following demonstrates the cooperative operation of such multiple actuators in an example embodiment having a plurality of processing modules, namely an enrichment zone 915, a microdroplet preparation module 158, a cell lysing ule 162 may be used to amplify and detect minute quantities 10 module 160, a mixing module 166 and a DNA manipulation module 167.

#### 1. Enrichment Module

a. Structure of Enrichment Module.

Referring to FIGS. 4 and 5, a microfluidic device 9§01 includes an enrichment module 156 for concentrating samples received therein. These samples include particlecontaining fluids, such as bacterial cell-containing fluids. In general, enrichment module 156 receives a flow of particlecontaining fluid from an input port 180 of input module 150, and allows the fluid to pass through the zone while accumulating particles within the zone. Thus, as more fluid flows through the zone, the particle concentration increases within the module. The resultant concentrated fluid sample is referred to herein as an enriched particle sample.

The enrichment module includes an enrichment zone 931 (FIG. 5), a flow through member 900, valves 915, 919, and sample introduction channel 929. Valve 919 is connected between the flow through member 900 and actuator 168 as shown, and valve 915 is connected between the flow through member and a down stream channel 937 which leads to process module 158. These valves may be of any type suitable for use in a microfluidic device, such as thermally actuated valves, as discussed in co-pending application Ser. No. 09/953,921, filed Sep. 9, 2001. The valves may be reversible between the open and closed states to allow reuse of enrichment module 931.

The flow through member is also connected to the sample input module 150 via the sample introduction channel 929 to allow fluid to flow into the enrichment zone. Valve 913 is connected to this sample introduction channel to control the in-flow and out-flow of fluid from the input port.

FIG. 5 is a cross-sectional view of the enrichment zone which shows the flow through member in greater detail. As shown, flow through member 900 has first and second surfaces 941, 943. First surface 941 is preferably adjacent enrichment chamber 931. Second surface 941 is preferably spaced apart from the enrichment chamber 931 by flow through member 900. Flow through member 900 is preferably formed of a material having pathways smaller than the diameter of the particles to be enriched, such as pores of less than about 2 microns in diameter, for example, about 0.45 microns. Suitable materials for constructing flow through member 900 include, for example, filter media such as paper or textiles, polymers having a network of pathways, and 55 glassy materials, such as glass frits.

FIGS. 6 and 7 depict cross sectional views of upper substrate 130 that illustrate an enrichment zone 931. As shown, fluid exits enrichment zone 931 through surface 941, passes through member 900 and enters a space 400. Space 400 may include an absorbent material 402 to absorb the exiting fluid. Thus, space 400 preferably provides a substantially self-contained region in which fluid exiting the enrichment zone can collect without contacting exterior portions of the microfluidic system 100.

Space 400 is formed during the fabrication of upper substrate 130. As discussed above, microfluidic features, such as zones and channels, are fabricated at surface 136 of substrate 7

130. Space 400, however, is fabricated at a surface 137, which is preferably disposed on the other side of substrate 130, opposite surface 136. Thus, even when surface 136 is mated with lower substrate 132, fluid can exit enrichment zone 931 via flow through member 900.

Flow through member 900 and absorbent material 402 do not require adhesives or other fasteners for positioning within substrate 130. Rather flow through member 900 and absorbent material 402 may be formed of a shape and size that substantially corresponds to space 400. Friction then holds flow through member 900 and absorbent material 402 in place once they are positioned in space 400. Any residual gap at locations 404 between flow through member 900 and substrate 130 should be small enough to prevent particles from exiting enrichment zone 931 through the gap 404. Naturally, 15 adhesive or other fastening means may be used to secure flow through member 900 or absorbent material 402.

In an alternative embodiment, a flow through member is formed integrally with a substrate by using microfabrication techniques, such as chemical etching, that introduce pores or other pathways into the substrate. The pores provide fluid passage between enrichment zone 931 and an outer portion of the substrate.

#### b. Operation of Enrichment Module

To enrich a sample, the device 901 operates as follows. 25 Referring to FIG. 4, valves 915, 919 are initially closed, and valve 913 is open. A particle-containing fluid is introduced into input port 180. Since valve 913 is open, it allows the sample to pass along channel 929 into enrichment zone 931. Alternatively, enrichment zone 931 can be configured to receive samples directly, such as by injection. Since valves 915 and 919 are closed, fluid is substantially prevented from escaping into actuator 977 and downstream channel 937.

Thus, flow through member 900 provides the only path for fluid to exit the enrichment channel. Fluid passes through 35 surface 941 and exits enrichment zone 931 via second surface 943, while particles accumulate within the zone. Enrichment zone 931 can therefore receive a volume of fluid that is larger than the volume of the enrichment chamber 931. Thus, as fluid flows through the chamber, the concentration of particles within the chamber increases relative to the concentration in the particle-containing fluid supplied at the sample input. Where the particles are cells, the concentration or number of cells in zone 931 preferably becomes great enough to perform a polymerase chain reaction (PCR) analysis of polynucleotides released from the cells in a downstream processing module.

Enrichment zone 931 thus prepares an enriched particle sample from particles of particle-containing fluids received therein. The enriched particle sample has a substantially 50 higher ratio of particles per volume of fluid (PPVF) than the corresponding ratio of the particle-containing fluid received by the enrichment zone. The PPVF of the enriched particle sample is preferably at least about 25 times, preferably about 250 times, more preferably about 1,000 times greater than the 55 PPVF of the particle-containing fluid.

After a sufficient volume of particle containing fluid has been received by enrichment zone 931, valve 913 is closed thereby blocking further flow of fluid into the enrichment zone, and preventing material in zone 931 from returning to the sample introduction port 180. Valves 915, 919 are then opened, preferably upon actuating heat sources associated therewith. When opened, valve 919 allows actuator 168 to push enriched sample, and valve 915 allows the enriched sample to move downstream.

Actuator 168 provides a motive force that moves the enriched particle sample from enrichment zone 931. Actuator

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168 is preferably a gas actuator, which provides a gas pressure upon actuation of a heat source 975, which is in thermal communication with a volume of gas 977. Actuation of heat source 975 raises the temperature and, therefore the pressure, of gas 977. The flow through member and the fluid therein substantially prevents gas from escaping the enrichment zone. Thus, the resulting gas pressure moves the enriched particle sample downstream from the enrichment zone 931.

The gas actuator may include elements to facilitate alternative pressure generation techniques such as chemical pressure generation. In another embodiment, the actuator may decrease a volume of gas associated with an upstream portion of the enrichment zone to thereby create a pressure differential across the sample that moves the sample from the enrichment zone. An example of such an element is a mechanical actuator, such as a plunger or diagram.

Rather than generating a positive pressure upstream from the enrichment zone, the gas actuator may decrease a pressure downstream from the zone relative to a pressure upstream. For example, the gas actuator may include a cooling element in thermal contact with a volume of gas associated with a downstream portion of the zone. Contraction of the gas upon actuating the cooling element creates a gas pressure difference between the upstream and downstream portions of the enrichment zone to move the enriched particle sample from the enrichment zone. Alternatively, a mechanical actuator may be used increase a volume of gas associated with a downstream portion of the enrichment zone to thereby decrease the pressure of the gas and move the enriched particle sample from the enrichment zone.

The enriched particle sample is preferably moved downstream with essentially no dilution thereof, i.e., the concentration of the enriched particles is not substantially decreased upon movement from the enrichment zone 931. Thus, removal of particles from the enrichment channel of the present invention does not require diluting or otherwise contacting the particles with a fluid different from the fluid of the particle-containing fluid introduced to the enrichment channel. In contrast, in systems that concentrate substances by surface adsorption, removal of the adsorbed substances requires an elution fluid, which contacts and thereby dilutes the substances.

Upon removal from the enrichment zone of the present invention, the enriched particle sample is preferably received by downstream channel 937. Downstream channel 937 leads to other processing modules, which perform further processing of the enriched particle sample. In the embodiment of FIG. 3, the enriched particle sample is received by a microdroplet preparation module 158, which prepares a microdroplet sample comprising a portion of the enriched particle sample.

#### 2. Microdroplet Preparation Module

#### a. Characteristics of a Microdroplet

A microdroplet **802** is a discrete sample having a predetermined volume between, for example, about 1.0 picoliter and about 0.5 microliters. Thus, microdroplets prepared by microdroplet preparation module provide a known amount of sample for further processing. The volume of the microdroplet prepared by the microdroplet preparation module is preferably essentially independent of the viscosity, electrical conductivity, and osmotic strength of the fluid of the microdroplet.

Microdroplet **802** is preferably defined by upstream and downstream boundaries each formed by a respective gas liquid interface **804**, **806**. The liquid of the interface is formed by

a surface of a liquid forming the microdroplet. The gas of the interface is gas present in the channels microfluidic of microfluidic device 901.

b. Structure and Operation of the Microdroplet Preparation Module

Referring to FIGS. 8a-8b and 9a-9b, microdroplet preparation module 158 prepares a microdroplet 802 from a microfluidic sample received therein. This module includes a microdroplet preparation zone 800, a positioning element 979, a gas actuator 170, and a valve 216 which cooperate to prepare microdroplet 800 from microfluidic samples received from the enrichment zone.

As explained above, actuator 168 of the enriched zone pushes the enriched sample into the microdroplet preparation zone 800. The enriched sample moves until reaching positioning element 979. In general, a positioning element inhibits the downstream progress of a microfluidic sample to thereby position the sample at a desired location. However, as explained more fully below, the positioning element does not 20 954. permanently inhibit progress of the sample. Rather, it allows the microfluidic sample to continue downstream at a predetermined later time.

The leading edge of microfluidic sample 808 that reaches positioning element 979 is positioned downstream from an 25 opening 820 of gas actuator 170. Accordingly, a first portion **821** of microfluidic sample **808** is disposed upstream from opening 820 and a second portion 822 of microfluidic sample 808 is disposed downstream from opening 820.

Referring to FIGS. 8a-8b, gas actuator 170 is actuated, 30 such as by DAQ 126, to thereby generate a gas pressure sufficient to separate microdroplet 802 from the second portion 822 of microfluidic sample 808. The gas pressure is preferably provided by the actuation of a heat source 958, which heats a volume of gas associated with gas actuator 957. 35 As the pressure increases, the gas expands, thereby separating a microdroplet 802 from the rest of sample 808. Microdroplet 802 may comprise only a portion, such as less than about 75%, or less than about 50%, of microfluidic sample 808 sions of microdroplet 802 are determined by the volume of the channel between fluid barrier 979 and opening 820. For example, for a channel having a uniform cross-sectional area, a length l<sub>1</sub> of microdroplet 802 corresponds to a distance d<sub>4</sub> between positioning element 979 and opening 820. Thus, a 45 microfluidic device can be configured to prepare microdroplets of any volume by varying the length between the fluid barrier and corresponding actuator opening.

Continued actuation of gas actuator 170 overcomes the inhibitory effect of positioning element 979, thereby driving 50 microdroplet 802 to a location downstream of microdroplet preparation zone 800 while the second portion 822 of the microfluidics sample moves upstream from microdroplet 802 to cell lysis module 160.

#### 3. Cell Lysis Module

Referring back to FIG. 3, a lysing module 160 receives the microdroplet 802 prepared by microdroplet preparation zone 800. In general, lysing module 160 releases material from inside the particles, such as by releasing intracellular material from cells.

As shown in FIGS. 4 and 12, lysing module 160 includes a lysing zone 950, a lysing mechanism within the lysing zone (such as electrodes 954), and a vented positioning element 200 positioned upstream from the lysing zone. The lysing mechanism preferably includes a set of electrodes or other 65 structures for generating electric fields within the lysing zone. The vented positioning element preferably includes a vent

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202, a valve 204, and a second positioning element 206 for inhibiting fluid from flowing into the vent.

As explained above, actuator 170 of the microdroplet preparation module 158 drives a microdroplet into cell lysis module 160. As the microdroplet moves into module 160, vented positioning element 200 positions microdroplet 802 in a lysing position with respect to electrodes 954. More specifically, as the microdroplet arrives in lysing module 160 it passes the opening of positioning element 200, because sec-10 ond positioning element 206 inhibits the microdroplet from flowing into vent 202. When the rear end of the microdroplet passes the opening of barrier 200, the propulsion gas from actuator 170 dissipates through vent 202, thereby substantially equalizing gas pressure upstream of microdroplet 802 with a pressure downstream of microdroplet 802. Thus, the microdroplet stops movement at a lysing position just downstream from barrier 200. Preferably, in the lysing position, substantially all of microdroplet 802 is disposed between an upstream edge 212 and a downstream edge 214 of electrodes

After microdroplet 802 is placed in the cell lysing position, a pulse circuit of DAQ 126 supplies a pulsed voltage signal across electrodes 954. In response, electrodes 954 generate a pulsed electric field in the vicinity of the electrodes. Because the microdroplet is position in this vicinity, cells within the microdroplet are subjected to the pulsed field. Preferably, substantially all of the cells, such as greater than about 75%, of the microdroplet are subjected to an electric field sufficient to release intracellular material therefrom. The lysing module thus prepares a lysed microdroplet comprising a predetermined amount of sample.

A preferred pulse circuit is shown in FIG. 14. In general, this circuit generates a sequence of voltage pulses that yields a corresponding sequence of electrical field pulses in the vicinity of electrodes 954 having an amplitude and duration sufficient to release a desired amount of intracellular material from cells within the microdroplet.

Intracellular material present in lysed microdroplet is accessible to further process steps. For example, DNA and/or received by microdroplet preparation zone 800. The dimen- 40 RNA released from cells is accessible for amplification by a polymerase chain reaction. As used herein, the term lysing does not require that the cells be completely ruptured. Rather, lysing refers to the release of intracellular material. For example, rather than rupturing the cells, the electric field may increase the porosity of cell membranes by an amount that allows release of intracellular material without permanent rupture of the membranes.

> Other lysing mechanisms may also be employed to release intracellular material from cells. For example, material may be released by subjecting cells to other forces including for example osmotic shock or pressure. Chemicals, selected from the group of surfactants, solvents, and antibiotics may be contacted with the cells. Mechanical shear methods may also be used to release intracellular materials.

The lysed microdroplet may be moved downstream to mixing module 160 for further processing. To move lysed microdroplet downstream, valve 216, which is disposed upstream of lysing zone 950, is closed. Valve 204 is also closed to prevent gas from exiting lysing zone 950 via vent. Actuator 60 170 is then actuated, as described above, to provide a gas pressure sufficient to move lysed microdroplet downstream of lysing zone 950.

In an alternative embodiment, a lysing module 300, as shown in FIGS. 13a, 13b, includes a lysing zone 302 which is configured to prepare a lysed microdroplet 304 of predetermined volume from a microfluidic sample 306, which may have an indeterminate volume. Lysing zone 302 preferably 11

includes a lysing mechanism such as electrodes 308. Electrical leads 310 provide a connection to a pulse circuit of DAQ 126, via contacts 112, chip carrier 120, and contacts 125. A positioning element 312 is disposed downstream of lysing zone 302. An actuator 314 is disposed upstream from lysing zone. Actuator 314 preferably includes a second positioning element 316 to prevent fluid from the microfluidic sample from entering therein.

Lysing zone 302 operates as follows. The microfluidic sample 306 enters lysing zone 302 and moves downstream until a downstream interface 316 of the microfluidic sample 306 encounters positioning element 312. The positioning element 312 preferably increases a surface tension of the downstream interface of the microfluidic sample 306, thereby inhibiting further downstream movement and positioning a portion of the microfluidic sample in a lysing position with respect to electrodes 308. The lysing position is defined as the location of the portion of the microfluidic sample disposed downstream of actuator 314 and upstream of positioning element 312. Preferably, actuator 314 and positioning element 312 are disposed adjacent electrodes 308 such that substantially all of the material present in the lysing position is subjected to the electric field upon actuating electrodes 308.

Actuation of electrodes 308 in the embodiment described 25 above, provides an electrical field sufficient to release intracellular material from cells present in the portion of the microfluidic sample in the lysing position. Once a sufficient amount of intracellular material has been released, actuator 314 is actuated to prepare lysed microdroplet 304 from the 30 microfluidic sample 306. Actuator 314 preferably provides a gas pressure sufficient to move the lysed microdroplet 304 to a downstream portion of a microfluidic device such as mixing module 166.

#### 4. Mixing Module And Reagent Input Module

Referring back to FIG. 4, a lysed sample prepared by lysing module 160 is received by mixing module 166. Mixing module 166 includes a mixing zone 958. In this zone, the lysed cell sample is contacted, such as by mixing, with an amount of reagent received from the reagent source module 152. 40 Reagent source module 152 includes a reagent microdroplet preparation zone (RMPZ) 434, which preferably operates to prepare a microdroplet having a predetermined volume of reagent.

#### a. Reagent Input Module

Reagentinput module 152 is essentially the same as microdroplet formation module 158, however, it is specifically designed for formation of a microdroplet of reagent having a predetermined volume which will yield a desired ratio of reagent to sample when mixed with the microdroplet from cell lysing module 160. Module 152 includes an input port 420, a valve 422, and an actuator 172, each of which joins a reagent source channel 428. An overflow channel 424, which also joins reagents source channel 428, may also be provided. Actuator 172 may include a second positioning element 432 55 to prevent liquid from entering therein.

Reagent materials, which preferably comprise at least one liquid, are introduced via input port 420, such as with a pipette or syringe. Examples of suitable reagent materials include substances to facilitate further processing of the lysed cell sample, such as enzymes and other materials for amplifying DNA therein by polymerase chain reaction (PCR). The reagent material moves downstream within reagent source channel 428 until a downstream portion of the reagent material contacts a positioning element 426. Any additional 65 reagent material that continues to be received within reagent source module preferably enters overflow channel 424. When

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the introduction of reagent is complete, valve 422 is closed to prevent reagent from exiting reagent source channel via reagent source port 420.

#### b. Mixing Module

Mixing zone 958 of the mixing module includes adjoined first and second channels 410, 412. Materials moving downstream toward mixing zone 958 contact one another and preferably mix therein. Because of the micro-scale dimensions of mixing zone 958, the sample and reagent materials preferably
 mix by diffusion even in the absence of other sources of mass transport, such as mechanical agitation. It should be understood however, that agitation forces, such as acoustic waves may be applied to enhance mixing within mixing zone 958.

c. Operation of Mixing Module and Reagent Input Module
Reagent source module 152 and mixing module 166 preferably operate as follows. When a lysed sample from lysing
zone 950 is ready to be mixed with reagent material, actuator
172 is actuated to prepare a microdroplet of reagent. The
microdroplet of reagent is prepared from the portion of
reagent material downstream of an opening 430 of actuator
172 and upstream of positioning element 427. Thus, assuming that the dimensions of the reagent source channel 428 are
constant, the volume of the microdroplet of reagent is determined by the distance between the positioning element 426
and the actuator opening 430.

The microdroplet of reagent moves downstream toward channel 412 of reagent mixing zone. Meanwhile, a sample of lysed material, such as a lysed microdroplet, is moved downstream from lysing zone 950 toward channel 410 of mixing zone 958. Actuator 170 may provide the motive force to move the lysed microdroplet downstream. Alternatively, as discussed above, another actuator may be disposed upstream of lysing zone 950 but downstream of actuator 170 to provide the necessary motive force.

The sample and reagent material enter a downstream channel 438 of mixing zone 958, where the materials contact and mix. Because both the lysed sample and reagent material are mixed in the form of microdroplets, mixing zone 958 prepares an amount of mixed material having a predetermined ratio of sample to reagent. The volumes of microdroplets prepared within microfluidic device 110 are preferably independent of physical properties, such as viscosity, electrical conductivity, and osmotic strength, of the microdroplets. Thus, mixing zone 958 prepares an amount of mixed material having a sample to reagent material that is also independent of the physical and chemical properties of the mixed materials. A vent 440, which is downstream of the various zones of the microfluidic device 110 ensures that downstream pressure buildup does not inhibit downstream movement of samples within microfluidic device 110.

#### 5. DNA Manipulation Module

The mixed lysed cell sample and reagent are received within a DNA manipulation zone 971 of DNA manipulation module 162. Module 162 can perform, for example, restriction, digestion, ligation, hybridization and amplification of DNA material. In one embodiment, DNA manipulation zone 971 is configured to perform PCR amplification of nucleic acids present within the lysed cell sample. Vent 440 prevents pressure from increasing within zone 971 as the lysed cell sample and reagent are being introduced thereto. Valves 972 and 973 of DNA manipulation module 162 may be closed to prevent substances therein zone from exiting, such as by evaporation, during PCR amplification. The DNA manipulation zone is configured with heat sources under control of computer 127 to allow thermal cycling of DNA manipulation zone during amplification, as understood by one of skill in the art.

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System 901 includes also includes a detector 981 to detect the presence of amplified polynucleotides produced by PCR. Detector 981 is preferably an optical detector in optical communication, such as by a fiber optic 981, with zone 971. A light source, such as a laser diode, introduces light to DNA Manipulation zone 971 to generate fluorescence indicative of the amount of amplified polynucleotides present therein. The fluorescence arises from fluorescent tags, included in the reagent and associated with the polynucleotides upon amplification.

C. Preferred Positioning Elements
Preferred positioning elements are discussed below.

#### 1. Non-Wetting Positioning Elements

A positioning element 979 may be formed by a non-wetting material disposed to contact a microfluidic sample. The physio-chemical properties of the non-wetting material are chosen upon considering the type of liquid forming the microfluidic sample. For example, where the microfluidic sample is an aqueous sample, the positioning element preferably comprises a hydrophobic material. An exemplary hydrophobic material includes a non-polar organic compound, such as an aliphatic silane, which can be formed by modifying an internal surface of microfluidic device 901. For microfluidic samples formed of organic solvents, the non-wetting material may comprise a hydrophilic material.

When microfluidic sample **808** encounters positioning element **979**, the liquid of the microfluidic sample experiences an increased surface tension at downstream interface **810**, which increased surface tension inhibits continued downstream motion of microfluidic sample **808**. Increasing the gas pressure difference between upstream and downstream portions of the microfluidic sample overcomes the resistance and moves the microfluidic sample downstream.

#### 2. Capillary Assisted Positioning Elements

Referring to FIGS. 10a-10c, another type of positioning element may be formed by modifying the dimensions of the 40 microfluidic channel to form a capillary assisted positioning element (CAFB) 700. A CAFB comprises an upstream feed zone 702, a loading zone 704, and a stop zone 704. A microfluidic sample 720 encountering the CAFB moves downstream until a downstream interface 710 of the microfluidic 45 sample contacts upstream surfaces 714 of the loading zone 706. At this point, capillary action causes the microfluidic sample to move downstream until the downstream sample interface 710 encounters the opening 712 between the loading zone 704 and the stop zone 706. Surface tension resists the 50 tendency of the microfluidic sample to continue downstream past opening 714. Thus, the microfluidic sample 720 is positioned at a predetermined location along the channel axis with respect to positioning element 700.

The volume of the microfluidic sample encountering the 55 CAFB preferably has a larger volume than a volume of the loading zone **704** to ensure that the microfluidic sample will advance fully to opening. For fluids that have similar surface tensions and interface properties as water, the depth d<sub>1</sub> of the loading zone **704** is preferably about 50% or less of the 60 respective depths d<sub>2</sub>, d<sub>3</sub> of the stop and feed zones.

The tendency of a microfluidic sample to move in a given direction is governed by the ratio between the mean radius of curvature (MRC) of the front of the microfluidic sample and the MRC of the back of the microfluidic sample. These curvatures depend upon the contact angle of the fluid of the sample and the dimensions of the zone in which the micro-

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droplet is moving. A MRC  $r_1$  of a microdroplet interface in the loading zone is preferably smaller than a MRC  $r_2$  of a droplet interface within the feed zone or a MRC  $r_3$  of a droplet interface within the stop zone. The MRC  $r_2$  is preferably larger than the MRC  $r_3$ . Thus, the radius of curvature of the downstream microdroplet interface increases upon encountering the stop zone thereby inhibiting further downstream movement. Preferably, the contact angle of the fluid with the wall is substantially constant throughout the capillary assisted loading zone.

#### 3. Vented Positioning Elements

Referring to FIGS. 11a-11c, a positioning element 500 operates to position a microfluidic sample 502 by reducing the gas pressure acting upon an upstream portion 504 of the microfluidic sample relative to the gas pressure acting upon a downstream portion 506 of the microfluidic sample. Positioning element 500 includes a vent 508 disposed in gaseous communication with a zone 510 along which microfluidic sample 502 moves. Vent 508 preferably communicates with zone 510 via a passage 526. The zone may be for example, a channel or conduit. Positioning element 500 may also include a second positioning element 516, such as a non-wetting material, to substantially prevent fluid from the microfluidic sample from contacting the vent.

An open state of a valve 512 allows passage of gas between zone 510 and vent 508. A closed state of valve 512 prevents such passage of gas. Valve 514 is preferably thermally actuated and includes a mass 514 of TRS.

An actuator 518 is disposed upstream of positioning element 500. Actuator 518 is preferably a gas actuator and may include a heat source 520 to heat a gas associated with actuator 518. Actuator 518 may include a positioning element 522, such as non-wetting material, to substantially prevent fluid from the microfluidic sample from entering therein.

Positioning element **500** preferably operates as follows. Referring to FIG. **11***a*, microfluidic sample **502** moves downstream in the direction of arrow **524**. Microfluidic sample is preferably moved by a gas pressure provided from an upstream actuator, which is not shown in FIGS. **9***a***-9***c*. The gas pressure acts upon upstream portion **504**.

Referring to FIG. 11b, when upstream portion 504 passes the opening of vent 508, the upstream gas dissipates through vent 508, thereby reducing the upstream pressure. The pressure reduction, which preferably equalizes the downstream and upstream pressures, reduces or eliminates the motive force tending to urge the microfluidic sample downstream.

Referring to FIG. 11c, valve 512 is closed to prevent passage of gas between zone 510 and vent 508. Preferably, TRS 514 moves into passage 526. Upon closing valve 512, the actuation of actuator 518 provides a motive force to move microfluidic sample 502 downstream in the direction of arrow 528 for further processing.

#### 4. Active Fluid Positioning Elements

Referring to FIGS. 15*a*-15*c*, a microdroplet preparation module 652 has a microdroplet preparation zone 650, an active fluid positioning element 654, an actuator 656, and a valve 658. A second actuator 660 is operatively associated with the active positioning element 654 to introduce a microfluidic sample 666 to the microdroplet preparation zone 650. Second actuator 660 is preferably located upstream from valve 658. Microdroplet preparation module 652 prepares a microdroplet 668, which has a predetermined volume from the microfluidic sample 666 received therein.

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In operation, microfluidic preparation module **652** receives the microfluidic sample **666**, which moves downstream because of a motive force provided by the second actuator **660**. The motive force is preferably an upstream gas pressure, which is greater than a downstream gas pressure acting upon the microfluidic sample **666**. The microfluidic sample moves downstream until a downstream portion **670** thereof encounters active positioning element **654**, which preferably comprises a sensor **672** having electrical leads **674**. The leads **674** are in electrical communication with I/O pins of the microfluidic device to allow signals from sensor **672** to be received by a DAO.

Sensing element 672 is preferably a pair of electrical contacts. To sense the presense of the liquid, DAQ 126 applies a small voltage across leads 674 and measures the resultant current. As the liquid of the microfluidic sample contacts the first and second contacts, the current passing therebetween changes, thereby indicating to DAQ 126 that the liquid has arrived at sensor 672.

Upon recognition that the liquid has arrived at sensor 672, the DAQ instructs second actuator 660 to decrease a downstream motive force acting upon the microfluidic sample 666. For example, DAQ may reduce a current flowing through a heat source 676 associated with second actuator 660 thereby reducing a temperature of a gas therein. The temperature reduction reduces the gas pressure acting upon a upstream portion 678 of microfluidic sample thereby inhibiting the downstream motion of the microfluidic sample 666. The microfluidic sample is positioned such that a first portion 680 is located downstream of actuator 656 and a second portion 682 is located upstream of actuator 656.

To prepare microdroplet 668, DAQ 126 actuates actuator to provide a motive force which prepares the microdroplet 668 from the first portion 680 of microfluidic sample 666. Microdroplet 668 moves downstream while the second portion 682 of the microfluidic sample 666 moves upstream from actuator 656. During microdroplet preparation, valve 658 may be closed to substantially isolate the actuator 656 from second actuator 660 and other upstream portions of the microfluidic device.

The active positioning element preferably operates as a closed loop element that provides feedback from sensor **672** to the DAQ. The feedback is indicated when a microfluidic sample has reached a predetermined position within the microfluidic device. Upon receiving the feedback, the DAQ changes the state of the actuator providing the motive force to move the microdroplet.

While the above invention has been described with reference to certain preferred embodiments, it should be kept in mind that the scope of the present invention is not limited to these. Thus, one skilled in the art may find variations of these preferred embodiments which, nevertheless, fall within the spirit of the present invention, whose scope is defined by the claims set forth below.

What is claimed is:

- 1. A system, comprising:
- a microfluidic device;
- a computer-controlled heat source; and
- a detector;

wherein the microfluidic device comprises:

- an upstream channel:
- a DNA manipulation module located downstream from the upstream channel;
- a DNA manipulation zone within the DNA manipulation module and configured to perform PCR amplification of a sample;
- a first valve disposed within the DNA manipulation module upstream of the DNA manipulation zone;

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- a second valve disposed within the DNA manipulation module downstream of the DNA manipulation zone; and
- a vent disposed within the DNA manipulation module and separated from the upstream channel by the first and second valves;
- a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the DNA manipulation zone when amplification of the sample occurs, wherein the only ingress to and egress from the DNA manipulation zone is through the first and second valves, and wherein the computer-controlled heat source is in thermal contact with the DNA manipulation zone; and
- wherein the detector is configured to identify one or more polynucleotides within the DNA manipulation zone.
- 2. The microfluidic device of claim 1, wherein the DNA manipulation zone is configured to receive a microdroplet of lysed cell sample and reagent fluid from the upstream channel
- 3. The microfluidic device of claim 2, wherein the microfluidic device is configured to subject the lysed cell sample and reagent fluid to a polymerase chain reaction thereby providing amplified polynucleotides.
- 4. The microfluidic device of claim 3, wherein the detector comprises a light source configured to introduce light to the DNA manipulation zone, the light selected to generate fluorescence indicative of the amount of amplified polynucleotides present therein.
- 5. The microfluidic device of claim 1, wherein the detector is an optical detector in optical communication with the DNA manipulation zone.
- **6**. The microfluidic device of claim **1**, wherein the vent is configured to prevent pressure from increasing within the DNA manipulation zone.
- 7. The microfluidic device of claim 1, wherein the computer-controlled heat source is configured to control thermal cycling of the DNA manipulation zone.
- vice.

  8. The microfluidic device of claim 2 further comprising an actuator configured to move a microdroplet of lysed cell sample into the DNA manipulation zone.
  - 9. The microfluidic device of claim 8, wherein the actuator is a gas actuator, and the device is configured to move the lysed cell sample and reagent fluid from the upstream channel to the DNA manipulation zone by opening the first valve and actuating the gas actuator to thereby increase a gas pressure within the upstream channel relative to a gas pressure within the DNA manipulation zone.
  - 10. The microfluidic device of claim 8, wherein the actua-50 tor is a gas actuator, and the device is configured to move the lysed cell sample and reagent fluid from the upstream channel to the DNA manipulation zone by opening the first valve and actuating the gas actuator to thereby decrease a gas pressure within the DNA manipulation zone relative to a gas pressure 55 within the upstream channel.
    - 11. The microfluidic device of claim 1, wherein the first and second valves comprise a thermally responsive substance.
  - 12. The microfluidic device of claim 11, wherein the first 60 and second valves are thermally actuated.
    - 13. The microfluidic device of claim 12, wherein the first and second valves are reversible between an open and a closed state.
    - 14. The microfluidic device of claim 13, wherein the first and second valves are configured, when in the closed state, to prevent gas and liquid within the DNA manipulation zone from entering or exiting the DNA manipulation zone.

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- 15. The microfluidic device of claim 1, wherein the device further comprises a lower substrate and an upper substrate, and the DNA manipulation zone, first and second valves, and vent are integral with the upper substrate.
- 16. The microfluidic device of claim 15, wherein the computer-controlled heat source comprises a plurality of resistive heaters.
- 17. The microfluidic device of claim 16, wherein the lower substrate has a glass base and an oxide layer, wherein the oxide layer contains the plurality of resistive heaters, and wherein the upper substrate has a bottom surface bonded to the oxide layer on the lower substrate.
  - **18**. A device, comprising:
  - a microfluidic process module;
  - a computer-controlled heat source; and
  - wherein the microfluidic process module comprises:
    - a zone configured to receive a sample and perform amplification of the sample;
    - a first valve upstream of the zone;
    - a second valve downstream of the zone; and
  - a vent separated from the first valve by the second valve; a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out
  - of the zone when amplification of the sample occurs in the zone, wherein the only ingress to and egress from the zone is through the first and second valves;
  - wherein the computer-controlled heat source is in thermal contact with the zone; and
  - wherein the detector is configured to identify one or more polynucleotides within the zone.
  - 19. A system, comprising:
  - a microfluidic device;
  - a computer-controlled heat source; and
  - a detector;

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wherein the microfluidic device comprises:

- an upstream channel;
- a DNA manipulation zone located downstream from the upstream channel and configured to perform PCR amplification of a sample;
- a first valve disposed upstream of the DNA manipulation zone: and
- a second valve disposed downstream of the DNA manipulation zone;
- a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the DNA manipulation zone and to isolate and confine the sample to a region between the first and second valves accessible to the detector, wherein the only ingress to and egress from the region accessible to the detector is through the first and second valves; and
- wherein the computer-controlled heat source is in thermal contact with the DNA manipulation zone and wherein the detector is configured to identify one or more polynucleotides within the DNA manipulation zone.
- 20. The system of claim 18, wherein the first and second valves comprise a thermally responsive substance.
- 21. The system of claim 18, wherein the first and second valves are thermally actuated.
- 22. The system of claim 18, wherein the first and second valves are reversible between an open and a closed state.
- 23. The system of claim 19, wherein the first and second valves comprise a thermally responsive substance.
- 24. The system of claim 19, wherein the first and second 30 valves are thermally actuated.
  - **25**. The system of claim **19**, wherein the first and second valves are reversible between an open and a closed state.

\* \* \* \* \*

# EXHIBIT 2



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Apr. 22, 2014

### (12) United States Patent

#### Handique et al.

#### (54) MOVING MICRODROPLETS IN A MICROFLUIDIC DEVICE

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- (63) Continuation of application No. 11/929,971, filed on Oct. 30, 2007, now Pat. No. 8,273,308, which is a continuation of application No. 10/075,371, filed on Feb. 15, 2002, now Pat. No. 7,323,140, which is a continuation-in-part of application No. 10/014,519, filed on Dec. 14, 2001, now Pat. No. 7,192,557, and a continuation-in-part of application No. 09/953,921, filed on Sep. 18, 2001, now Pat. No. 6,575,188, and a continuation-in-part of application No. 09/819,105, filed on Mar. 28, 2001, now Pat. No. 7,010,391.
- Provisional application No. 60/307,638, filed on Jul. 26, 2001.
- (51) **Int. Cl.** G01N 15/06 (2006.01)
- U.S. Cl. (52)

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Field of Classification Search

USPC ...... 422/68.1, 81, 82, 100, 502, 503, 504, 422/509; 436/43, 174, 180

See application file for complete search history.

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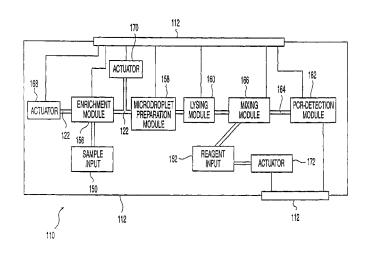
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#### (57)ABSTRACT

This disclosure provides systems, methods, and devices for processing samples on a microfluidic device. One method includes moving a sample from an upstream channel of a microfluidic device into a DNA manipulation module located downstream of the upstream channel. The DNA manipulation module includes a DNA manipulation zone configured to perform amplification of the sample, a first valve disposed upstream of the DNA manipulation zone, and a second valve disposed downstream of the DNA manipulation zone. The method also includes receiving the sample in the DNA manipulation zone; closing the first valve and the second valve such that as and liquid are prevented from flowing into or out of the DNA manipulation zone; and thermal cycling the sample in the DNA manipulation zone.

#### 10 Claims, 15 Drawing Sheets



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# MOVING MICRODROPLETS IN A MICROFLUIDIC DEVICE

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of application Ser. No. 11/929,971, filed Oct. 30, 2007, which is a continuation of application Ser. No. 10/075,371, filed Feb. 15, 2002, which is a continuation-in-part of application Ser. No. 10/014,519, filed Dec. 14, 2001, application Ser. No. 09/953,921, filed Sep. 18, 2001, and application Ser. No. 09/819,105, filed Mar. 28, 2001, and claiming priority of provisional application No. 60/307,638 filed Jul. 26, 2001. Each of the above-mentioned applications are incorporated herein by reference.

#### BACKGROUND OF THE INVENTION

#### 1. Field of the Invention

The present invention relates to methods and systems for processing samples using microfluidic systems. More particularly, the invention relates to moving fluid samples within a microfluidic system. Description of the Related Art

#### 2. Background

Microfluidic devices are typically formed of substrates (made of silicon, glass, ceramic, plastic and/or quartz) which include a network of micro-channels through which fluid flows under the control of a propulsion mechanism. The micro channels typically have at least one dimension which is on the order of nanometers to hundreds of microns.

Microfluidic devices process minute amounts of fluid sample to determine the physical and chemical properties of the sample. Microfluidic devices offer several advantages over a traditional macro-scale instrumentation. For example, in general, they require substantially smaller fluid samples, use far less reagent, and process these fluids at substantially greater speeds than macro-scale equipment.

Electric fields are used as a propulsion mechanism for some microfluidic devices. In such devices, a high voltage, on the order of kilovolts, is applied across electrodes within the device to thereby generate an electric field in the micro channels. The field imposes a force on ions within the fluid, thereby propelling the ions through the micro channel. The fluid itself may also be propelled by the motion of ions moving within the fluid.

Gas pressure is also used to propel fluid through micro channels. In some devices, a source of pressurized gas, external to the microfluidic device, is connected to the microfluidic device to supply a gas pressure, which propels the fluid. Gas pressure may also be generated by a heated chamber within the microfluidic device itself to propagate fluid within a micro channel.

#### SUMMARY OF THE INVENTION

In general, the invention relates to a system and method for moving samples, such as fluids, within a microfluidic system.

In one aspect, the invention relates to the use of a plurality of gas actuators for applying pressure at different locations within the microfluidic system to thereby supply force for moving samples. For example, in one embodiment, a first gas actuator provides a gas pressure sufficient to move a first sample from a first location to a second location of the microfluidic device. A second gas actuator provides a gas pressure to module for move another sample from a third location to a fourth location of the microfluidic device.

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In another example, a plurality of gas actuators cooperate to move the same fluid sample. A first gas actuator provides a gas pressure sufficient to move the microdroplet between first and second processing zones of the microfluidic device, and a second gas actuator provides a gas pressure to move the microdroplet to a third processing zone.

In preferred embodiments, the plurality of actuators are integral with a microfluidic network through which the microfluidic samples flow. For example, a plurality of gas actuators can be fabricated in the same substrate which forms the microfluidic network. One such gas actuator is coupled to the network at a first location for providing gas pressure to move a microfluidic sample within the network. Another gas actuator is coupled to the network at a second location for providing gas pressure to further move at least a portion of the microfluidic sample within the network.

In other aspect, the invention relates to the use of valves with the plurality of actuators. For example, in one embodiment, a valve is coupled to a microfluidic network so that, when the valve is closed, it substantially isolates the second gas actuator from the first gas actuator. Such valves can control the direction of the propulsive force of the actuatators by preventing the expanding gas from traveling in certain directions, while permitting it to expand in the desired direction. They also extend the range over which an actuator can propel a microdroplet, by preventing the gas from dissipating in certain in areas upstream from the microdroplet.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The present invention is described below in reference to the following drawings, in which:

FIG. 1 shows a microfluidic system according to the invention:

FIG. 2 shows an expanded view of a microfluidic device.

FIG. 3 shows a schematic of a microfluidic device of the microfluidic system of FIG.

FIG. 4 shows a top view of the microfluidic device of FIG. 3.

FIG. 5 shows a partial cross-sectional view of the microfluidic device of FIG. 4;

FIG. 6 shows a partial cross-sectional view of an upper substrate from the microfluidic device of FIG. 2;

FIG. 7 shows a second partial cross-sectional view of an upper substrate from the microfluidic device of FIG. 2;

FIG. 8a shows a top view of a microdroplet preparation zone of the microfluidic device of FIG. 4 before preparation of a microdroplet;

FIG. 8b shows cross sectional view of the microdroplet preparation zone of FIG. 8a;

FIG. 9a shows a top view of a microdroplet preparation zone of the microfluidic device of FIG. 4 after preparation of a microdroplet;

FIG. 9b shows a cross sectional side view of the micro-droplet preparation zone of FIG. 9a;

FIGS. 10a-10c show cross sectional side views of a capillary assisted fluid barrier of the present invention;

FIGS. 11a-11c show top views of a fluid barrier comprising a vent:

FIGS. 12a and 12b show top views of the lysing module of the microfluidic device of FIG. 4, before and after preparation of a lysed sample;

FIGS. 13a and 13b show a second embodiment of a lysing module of the invention;

FIG. 14 shows a pulsing circuit associated with the lysing module of FIG. 4; and

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FIGS. **15***a***-15***c* show a second microdroplet preparation module of the invention.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention relates to microfluidic systems and methods for processing materials, such as samples and reagents. More specifically, the invention relates to microfluidic systems and methods for moving fluids within a microfluidic system. In the embodiment described below, the fluid includes particles which tend to move with the fluid. The fluid component of the particle-containing fluid is a gas or, preferably, a liquid. The particles of the particle-containing fluid are preferably whole cells, such as bacterial cells or cells of an animal, such as a human. However, they may include intracellular material from such cells. For example, a system of the invention may be used to process a sample of bacterial cells to determine whether the bacteria are pathogenic.

#### A. System Overview

FIG. 1 depicts a microfluidic system 100 that includes a microfluidic device 110 and corresponding cartridge 120, which receive one or more fluid samples and process the samples under the control of computer 127 and data acquisition and control board (DAQ) 126.

Computer 127 preferably performs high level functions, such as supplying a user interface that allows a user to select 30 desired operations, notifying the DAQ 126 as to the selected operations, and displaying for the user the results of such operations. These operations include, for example, subjecting a sample to process steps within the various process zones of the microfluidic device. The computer 127 may be a portable 35 computer to facilitate transport of the microfluidic system.

Computer 127 is connected to DAQ 126 via connection 128, which provides data I/O, power, ground, reset, and other functional connectivity. Alternatively, a wireless link 132 between the computer 127 and the DAQ 126 may be provided 40 for data and control signal exchange via wireless elements 132(a) and 132(b). Where the data link is a wireless link, for example, the DAQ 126 may have separate power source, such as a battery.

In general, DAQ 126 controls the operation of microfluidic device 110 in accordance with the high level instructions received from computer 127. More specifically, to implement a desired operation requested by computer 127, DAQ 126 supplies the appropriate electrical control signals to cartridge 120 via contacts 125.

Cartridge 120 provides electrical and optical connections 121 for electrical and optical signals between the DAQ 126 and the microfluidic substrate 110, thereby allowing DAQ 126 to control the operation of the substrate.

The chip carrier cartridge 120 is shown being inserted into (or removed from) an interface hardware receptacle of the DAQ 126 having electrical and optical contacts 125 standardized to mate with a corresponding contacts 121 of the chip carrier cartridge 120. Most contacts are for electrical signals, 60 while certain ones are for optical signals (IR, visible, UV, etc.) in the case of optically-monitored or optically-excited microfluidic processors. Alternatively (not shown), the entire DAQ 126 may be a single ASIC chip that is incorporated into the Chip Carrier Cartridge 120, wherein contacts 121,125 65 would become conductive pathways on a printed circuit board.

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#### B. Microfluidic Device

FIG. 2 illustrates the general structure of a preferred type of microfluidic device. The device includes an upper substrate 130, which is bonded to a lower substrate 132 to form a fluid network.

The upper substrate 130 depicted in FIG. 2 is preferably formed of glass and has a microfluidic network 134 in its bottom surface 136. Those skilled in the art will recognize that substrates composed of silicon, glass, ceramic, plastic, and/or quartz are all acceptable in the context of the present invention.

The microfluidic network includes a plurality of zones. The number of zones, as well as the overall topology of the microfluidic network, will depend upon the particular application which the microfluidic device is designed to perform. The zones of the microfluidic device may have any cross-sectional shape, such as generally arcuate or generally polygonal. For example, a zone may include channels, chambers or other substantially enclosed spaces. By "substantially enclosed" it is meant that materials enter or exit the zones only through predetermined pathways. Examples of such pathways include channels, microchannels and the like, which interconnect the various zones. The zones preferably have at least one micro-scale dimension, such as less than about 250 µm or, more preferably, less than about 75 µm.

The channels and chambers of the microfluidic network are etched in the bottom surface 136 of the upper substrate 130 using known photolithographic techniques. More specifically, transparent templates or masks containing opaque designs are used to photo-define objects on the surface of the substrate. The patterns on the templates are generated with computer-aided-design programs and can delineate structures with line-widths of less than one micron. Once a template is generated, it can be used almost indefinitely to produce identical replicate structures. Consequently, even extremely complex microfluidic networks can be reproduced in mass quantities and at low incremental unit cost. Alternatively, if a plastic material is used, the upper substrate may be formed using injection molding techniques, wherein the micro-channels are formed during the molding process.

The lower substrate 132 may include a glass base 138 and an oxide layer 140. Within oxide layer 140, resistive heaters 142 and electric leads 144 are formed using photo-lithographic techniques. The leads 144 connect to terminals 146 which are exposed at the edge of the substrate to penult electrical connection to cartridge 120, thereby permitting DAQ 126 to control the heaters. More specifically, to activate a heater 142, DAQ 126 applies a voltage across a pair of terminals 146 (via cartridge 120) to supply current through leads 146 and heater 142, thereby heating the resistive heater element 142.

Metal heater elements 142 are positioned so that, when the upper and lower substrates are bonded together, the heaters reside directly beneath certain regions of the fluid network of the upper substrate so as to be able to heat the contents of these regions. The silicon oxide layer 140 prevents the heating elements 142 from directly contacting with material in the microfluidic network.

The oxide layer **140**, heating elements **142**, and resistive leads **144** are fabricated using well-known photolithographic techniques, such as those used to etch microfluidic network.

FIG. 3 illustrates a top-down view of microfluidic device 110. As shown, the substrate has a sample input module 150 and reagent input module 152 to allow sample and reagent materials, respectively, to be input to device 110. Preferably, input modules 150, 152 are disposed to allow automatic material input using a computer controlled laboratory robot 154.

The substrate also includes process modules 156, 158, 160, 166 and 162 for processing the sample and reagent materials. Within these process modules, a sample may be subjected to various physical and chemical process steps. For example, enrichment module 156 prepares a fluid sample having a 5 relatively high concentration of cell particles, lysing module 160 releases intracellular material from the cell particles, and mixing module 166 mixes the resultant sample with certain reagents. As another example, an amplification process module 162 may be used to amplify and detect minute quantities 10

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Various modules of microfluidic device 110 are connected, such as by channels 164, to allow materials to be moved from one location to another within the device 110. Actuators 168, 170, 172 associated with the microfluidic device provide a motive force, such as a gas pressure, to move the sample and reagent material along the channels and zones. For example, a first actuator 168 moves material downstream from process module 156 to process module 158. Upon completion of processing within process module 158, a second actuator 170 moves material downstream to mixing process module 160. Subsequently, actuator 170 or an additional actuator moves the material to mixing module 166, where the material mixes with a reagent moved by actuator 172. Finally, actuator 172, or another actuator, moves the mixed material to module 162.

Because each actuator is preferably responsible for moving materials within only a subset of the modules of device 110, sample materials can be controlled more precisely than if a single actuator were responsible for moving material throughout the entire device. The various functional elements, of microfluidic device 110, including the actuators, are preferably under computer control to allow automatic sample processing and analysis.

#### C. Multiple Actuators

of DNA within a sample.

The various actuators of microfluidic device 110 cooperate 35 to move material between different locations of microfluidic device 110. For example, actuator 168 moves material, such as an enriched sample, between an enrichment zone 931 and a microdroplet preparation module 158. Actuator 170 prepares a microdroplet from the enriched sample and, in so 40 doing, moves the microdroplet to a lysing zone 950. Actuator 170 is used to move material from the lysing zone 950 to mixing module 166. It should be noted, however, that another actuator may be disposed intermediate between lysing zone 950 and microdroplet preparation zone to move the lysed 45 sample downstream to the mixing module 166.

Actuators of device 110 may also cooperate in moving two amounts of material simultaneously. For example, as described above, actuator 172 and actuator 170 cooperate to mix reagent and lysed microdroplets. Such cooperative actuators can be controlled independently of one another to ensure proper mixing. For example, if one material is known to be more viscous, the motive force moving that material can be increased independently of the motive force moving the other material.

The multiple actuators and modules of microfluidic device 110 are preferably operatively connectable and isolatable by the valves of microfluidic device. For example, a closed state of either of valves 915, 216 operatively isolates microdroplet preparation module 170 from enrichment module 156. Thus, 60 one or more actuators can be used to move materials between predetermined locations within microfluidic device 110, without perturbing or contacting material present in an operatively isolated module. The ability to operatively connect and isolate desired modules is advantageous in microfluidic 65 devices having many process functions. Further, these valves also control the direction of the propulsive force of the actua-

tators by preventing the expanding gas from traveling in certain directions, while permitting it to expand in the desired direction. This also extends the range over which an actuator can propel a microdroplet, by preventing the gas from dissi-

The following demonstrates the cooperative operation of such multiple actuators in an example embodiment having a plurality of processing modules, namely an enrichment zone 915, a microdroplet preparation module 158, a cell lysing module 160, a mixing module 166 and a DNA manipulation module 167.

pating in certain in areas upstream from the microdroplet.

- 1. Enrichment Module
- a. Structure of Enrichment Module.

Referring to FIGS. 4 and 5, a microfluidic device 9.sctn.01 includes an enrichment module 156 for concentrating samples received therein. These samples include particle-containing fluids, such as bacterial cell-containing fluids. In general, enrichment module 156 receives a flow of particle-containing fluid from an input port 180 of input module 150, and allows the fluid to pass through the zone while accumulating particles within the zone. Thus, as more fluid flows through the zone, the particle concentration increases within the module. The resultant concentrated fluid sample is referred to herein as an enriched particle sample.

The enrichment module includes an enrichment zone 931 (FIG. 5), a flow through member 900, valves 915, 919, and sample introduction channel 929. Valve 919 is connected between the flow through member 900 and actuator 168 as shown, and valve 915 is connected between the flow through member and a down stream channel 937 which leads to process module 158. These valves may be of any type suitable for use in a microfluidic device, such as thermally actuated valves, as discussed in co-pending application Ser. No. 09/953,921, filed Sep. 9, 2001. The valves may be reversible between the open and closed states to allow reuse of enrichment module 931.

The flow through member is also connected to the sample input module 150 via the sample introduction channel 929 to allow fluid to flow into the enrichment zone. Valve 913 is connected to this sample introduction channel to control the in-flow and out-flow of fluid from the input port.

FIG. 5 is a cross-sectional view of the enrichment zone which shows the flow through member in greater detail. As shown, flow through member 900 has first and second surfaces 941, 943. First surface 941 is preferably adjacent enrichment chamber 931. Second surface 941 is preferably spaced apart from the enrichment chamber 931 by flow through member 900. Flow through member 900 is preferably formed of a material having pathways smaller than the diameter of the particles to be enriched, such as pores of less than about 2 microns in diameter, for example, about 0.45 microns. Suitable materials for constructing flow through member 900 include, for example, filter media such as paper or textiles, polymers having a network of pathways, and glassy materials, such as glass frits.

FIGS. 6 and 7 depict cross sectional views of upper substrate 130 that illustrate an enrichment zone 931. As shown, fluid exits enrichment zone 931 through surface 941, passes through member 900 and enters a space 400. Space 400 may include an absorbent material 402 to absorb the exiting fluid. Thus, space 400 preferably provides a substantially self-contained region in which fluid exiting the enrichment zone can collect without contacting exterior portions of the microfluidic system 100.

Space 400 is formed during the fabrication of upper substrate 130. As discussed above, microfluidic features, such as zones and channels, are fabricated at surface 136 of substrate

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130. Space 400, however, is fabricated at a surface 137, which is preferably disposed on the other side of substrate 130, opposite surface 136. Thus, even when surface 136 is mated with lower substrate 132, fluid can exit enrichment zone 931 via flow through member 900.

Flow through member 900 and absorbent material 402 do not require adhesives or other fasteners for positioning within substrate 130. Rather flow through member 900 and absorbent material 402 may be formed of a shape and size that substantially corresponds to space 400. Friction then holds 10 flow through member 900 and absorbent material 402 in place once they are positioned in space 400. Any residual gap at locations 404 between flow through member 900 and substrate 130 should be small enough to prevent particles from exiting enrichment zone 931 through the gap 404. Naturally, 15 adhesive or other fastening means may be used to secure flow through member 900 or absorbent material 402.

In an alternative embodiment, a flow through member is formed integrally with a substrate by using microfabrication techniques, such as chemical etching, that introduce pores or 20 other pathways into the substrate. The pores provide fluid passage between enrichment zone **931** and an outer portion of the substrate.

#### b. Operation of Enrichment Module

To enrich a sample, the device 901 operates as follows. 25 Referring to FIG. 4, valves 915, 919 are initially closed, and valve 913 is open. A particle-containing fluid is introduced into input port 180. Since valve 913 is open, it allows the sample to pass along channel 929 into enrichment zone 931. Alternatively, enrichment zone 931 can be configured to 30 receive samples directly, such as by injection. Since valves 915 and 919 are closed, fluid is substantially prevented from escaping into actuator 977 and downstream channel 937.

Thus, flow through member 900 provides the only path for fluid to exit the enrichment channel. Fluid passes through 35 surface 941 and exits enrichment zone 931 via second surface 943, while particles accumulate within the zone. Enrichment zone 931 can therefore receive a volume of fluid that is larger than the volume of the enrichment chamber 931. Thus, as fluid flows through the chamber, the concentration of particles within the chamber increases relative to the concentration in the particle-containing fluid supplied at the sample input. Where the particles are cells, the concentration or number of cells in zone 931 preferably becomes great enough to perform a polymerase chain reaction (PCR) analysis of polynucleotides released from the cells in a downstream processing module.

Enrichment zone **931** thus prepares an enriched particle sample from particles of particle-containing fluids received therein. The enriched particle sample has a substantially 50 higher ratio of particles per volume of fluid (PPVF) than the corresponding ratio of the particle-containing fluid received by the enrichment zone. The PPVF of the enriched particle sample is preferably at least about 25 times, preferably about 250 times, more preferably about 1,000 times greater than the 55 PPVF of the particle-containing fluid.

After a sufficient volume of particle containing fluid has been received by enrichment zone 931, valve 913 is closed thereby blocking further flow of fluid into the enrichment zone, and preventing material in zone 931 from returning to 60 the sample introduction port 180. Valves 915, 919 are then opened, preferably upon actuating heat sources associated therewith. When opened, valve 919 allows actuator 168 to push enriched sample, and valve 915 allows the enriched sample to move downstream.

Actuator 168 provides a motive force that moves the enriched particle sample from enrichment zone 931. Actuator

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168 is preferably a gas actuator, which provides a gas pressure upon actuation of a heat source 975, which is in thermal communication with a volume of gas 977. Actuation of heat source 975 raises the temperature and, therefore the pressure, of gas 977. The flow through member and the fluid therein substantially prevents gas from escaping the enrichment zone. Thus, the resulting gas pressure moves the enriched particle sample downstream from the enrichment zone 931.

The gas actuator may include elements to facilitate alternative pressure generation techniques such as chemical pressure generation. In another embodiment, the actuator may decrease a volume of gas associated with an upstream portion of the enrichment zone to thereby create a pressure differential across the sample that moves the sample from the enrichment zone. An example of such an element is a mechanical actuator, such as a plunger or diagram.

Rather than generating a positive pressure upstream from the enrichment zone, the gas actuator may decrease a pressure downstream from the zone relative to a pressure upstream. For example, the gas actuator may include a cooling element in thermal contact with a volume of gas associated with a downstream portion of the zone. Contraction of the gas upon actuating the cooling element creates a gas pressure difference between the upstream and downstream portions of the enrichment zone to move the enriched particle sample from the enrichment zone. Alternatively, a mechanical actuator may be used increase a volume of gas associated with a downstream portion of the enrichment zone to thereby decrease the pressure of the gas and move the enriched particle sample from the enrichment zone.

The enriched particle sample is preferably moved downstream with essentially no dilution thereof, i.e., the concentration of the enriched particles is not substantially decreased upon movement from the enrichment zone 931. Thus, removal of particles from the enrichment channel of the present invention does not require diluting or otherwise contacting the particles with a fluid different from the fluid of the particle-containing fluid introduced to the enrichment channel. In contrast, in systems that concentrate substances by surface adsorption, removal of the adsorbed substances requires an elution fluid, which contacts and thereby dilutes the substances.

Upon removal from the enrichment zone of the present invention, the enriched particle sample is preferably received by downstream channel 937. Downstream channel 937 leads to other processing modules, which perform further processing of the enriched particle sample. In the embodiment of FIG. 3, the enriched particle sample is received by a microdroplet preparation module 158, which prepares a microdroplet sample comprising a portion of the enriched particle sample.

#### 2. Microdroplet Preparation Module

#### a. Characteristics of a Microdroplet

A microdroplet **802** is a discrete sample having a predetermined volume between, for example, about 1.0 picoliter and about 0.5 microliters. Thus, microdroplets prepared by microdroplet preparation module provide a known amount of sample for further processing. The volume of the microdroplet prepared by the microdroplet preparation module is preferably essentially independent of the viscosity, electrical conductivity, and osmotic strength of the fluid of the microdroplet.

Microdroplet **802** is preferably defined by upstream and downstream boundaries each formed by a respective gas liquid interface **804**, **806**. The liquid of the interface is formed by

a surface of a liquid forming the microdroplet. The gas of the interface is gas present in the channels microfluidic of microfluidic device **901**.

b. Structure and Operation of the Microdroplet Preparation Module

Referring to FIGS. 8a-8b and 9a-9b, microdroplet preparation module 158 prepares a microdroplet 802 from a microfluidic sample received therein. This module includes a microdroplet preparation zone 800, a positioning element 979, a gas actuator 170, and a valve 216 which cooperate to prepare microdroplet 800 from microfluidic samples received from the enrichment zone.

As explained above, actuator 168 of the enriched zone pushes the enriched sample into the microdroplet preparation zone 800. The enriched sample moves until reaching positioning element 979. In general, a positioning element inhibits the downstream progress of a microfluidic sample to thereby position the sample at a desired location. However, as explained more fully below, the positioning element does not permanently inhibit progress of the sample. Rather, it allows the microfluidic sample to continue downstream at a predetermined later time.

The leading edge of microfluidic sample **808** that reaches positioning element **979** is positioned downstream from an 25 opening **820** of gas actuator **170**. Accordingly, a first portion **821** of microfluidic sample **808** is disposed upstream from opening **820** and a second portion **822** of microfluidic sample **808** is disposed downstream from opening **820**.

Referring to FIGS. 8a-8b, gas actuator 170 is actuated, 30 such as by DAQ 126, to thereby generate a gas pressure sufficient to separate microdroplet 802 from the second portion 822 of microfluidic sample 808. The gas pressure is preferably provided by the actuation of a heat source 958, which heats a volume of gas associated with gas actuator 957. 35 As the pressure increases, the gas expands, thereby separating a microdroplet 802 from the rest of sample 808. Microdroplet 802 may comprise only a portion, such as less than about 75%, or less than about 50%, of microfluidic sample 808 received by microdroplet preparation zone **800**. The dimen- 40 sions of microdroplet 802 are determined by the volume of the channel between fluid barrier 979 and opening 820. For example, for a channel having a uniform cross-sectional area, a length 1.sub.1 of microdroplet 802 corresponds to a distance d.sub.4 between positioning element 979 and opening 820. 45 Thus, a microfluidic device can be configured to prepare microdroplets of any volume by varying the length between the fluid barrier and corresponding actuator opening.

Continued actuation of gas actuator 170 overcomes the inhibitory effect of positioning element 979, thereby driving 50 microdroplet 802 to a location downstream of microdroplet preparation zone 800 while the second portion 822 of the microfluidics sample moves upstream from microdroplet 802 to cell lysis module 160.

#### 3. Cell Lysis Module

Referring back to FIG. 3, a lysing module 160 receives the microdroplet 802 prepared by microdroplet preparation zone 800. In general, lysing module 160 releases material from inside the particles, such as by releasing intracellular material from cells.

As shown in FIGS. 4 and 12, lysing module 160 includes a lysing zone 950, a lysing mechanism within the lysing zone (such as electrodes 954), and a vented positioning element 200 positioned upstream from the lysing zone. The lysing mechanism preferably includes a set of electrodes or other 65 structures for generating electric fields within the lysing zone. The vented positioning element preferably includes a vent

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202, a valve 204, and a second positioning element 206 for inhibiting fluid from flowing into the vent.

As explained above, actuator 170 of the microdroplet preparation module 158 drives a microdroplet into cell lysis module 160. As the microdroplet moves into module 160, vented positioning element 200 positions microdroplet 802 in a lysing position with respect to electrodes 954. More specifically, as the microdroplet arrives in lysing module 160 it passes the opening of positioning element 200, because second positioning element 206 inhibits the microdroplet from flowing into vent 202. When the rear end of the microdroplet passes the opening of barrier 200, the propulsion gas from actuator 170 dissipates through vent 202, thereby substantially equalizing gas pressure upstream of microdroplet 802 with a pressure downstream of microdroplet 802. Thus, the microdroplet stops movement at a lysing position just downstream from barrier 200. Preferably, in the lysing position, substantially all of microdroplet 802 is disposed between an upstream edge 212 and a downstream edge 214 of electrodes

After microdroplet **802** is placed in the cell lysing position, a pulse circuit of DAQ **126** supplies a pulsed voltage signal across electrodes **954**. In response, electrodes **954** generate a pulsed electric field in the vicinity of the electrodes. Because the microdroplet is position in this vicinity, cells within the microdroplet are subjected to the pulsed field. Preferably, substantially all of the cells, such as greater than about 75%, of the microdroplet are subjected to an electric field sufficient to release intracellular material therefrom. The lysing module thus prepares a lysed microdroplet comprising a predetermined amount of sample.

A preferred pulse circuit is shown in FIG. 14. In general, this circuit generates a sequence of voltage pulses that yields a corresponding sequence of electrical field pulses in the vicinity of electrodes 954 having an amplitude and duration sufficient to release a desired amount of intracellular material from cells within the microdroplet.

Intracellular material present in lysed microdroplet is accessible to further process steps. For example, DNA and/or RNA released from cells is accessible for amplification by a polymerase chain reaction. As used herein, the term lysing does not require that the cells be completely ruptured. Rather, lysing refers to the release of intracellular material. For example, rather than rupturing the cells, the electric field may increase the porosity of cell membranes by an amount that allows release of intracellular material without permanent rupture of the membranes.

Other lysing mechanisms may also be employed to release intracellular material from cells. For example, material may be released by subjecting cells to other forces including for example osmotic shock or pressure. Chemicals, selected from the group of surfactants, solvents, and antibiotics may be contacted with the cells. Mechanical shear methods may also be used to release intracellular materials.

The lysed microdroplet may be moved downstream to mixing module 160 for further processing. To move lysed microdroplet downstream, valve 216, which is disposed upstream of lysing zone 950, is closed. Valve 204 is also closed to prevent gas from exiting lysing zone 950 via vent. Actuator
170 is then actuated, as described above, to provide a gas pressure sufficient to move lysed microdroplet downstream of lysing zone 950.

In an alternative embodiment, a lysing module 300, as shown in FIGS. 13a, 13b, includes a lysing zone 302 which is configured to prepare a lysed microdroplet 304 of predetermined volume from a microfluidic sample 306, which may have an indeterminate volume. Lysing zone 302 preferably

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includes a lysing mechanism such as electrodes **308**. Electrical leads **310** provide a connection to a pulse circuit of DAQ **126**, via contacts **112**, chip carrier **120**, and contacts **125**. A positioning element **312** is disposed downstream of lysing zone **302**. An actuator **314** is disposed upstream from lysing zone. Actuator **314** preferably includes a second positioning element **316** to prevent fluid from the microfluidic sample from entering therein.

Lysing zone 302 operates as follows. The microfluidic sample 306 enters lysing zone 302 and moves downstream until a downstream interface 316 of the microfluidic sample 306 encounters positioning element 312. The positioning element 312 preferably increases a surface tension of the downstream interface of the microfluidic sample 306, thereby inhibiting further downstream movement and positioning a portion of the microfluidic sample in a lysing position with respect to electrodes 308. The lysing position is defined as the location of the portion of the microfluidic sample disposed downstream of actuator 314 and upstream of positioning element 312. Preferably, actuator 314 and positioning element 312 are disposed adjacent electrodes 308 such that substantially all of the material present in the lysing position is subjected to the electric field upon actuating electrodes 308.

Actuation of electrodes 308 in the embodiment described above, provides an electrical field sufficient to release intracellular material from cells present in the portion of the microfluidic sample in the lysing position. Once a sufficient amount of intracellular material has been released, actuator 314 is actuated to prepare lysed microdroplet 304 from the microfluidic sample 306. Actuator 314 preferably provides a gas pressure sufficient to move the lysed microdroplet 304 to a downstream portion of a microfluidic device such as mixing module 166.

#### 4. Mixing Module and Reagent Input Module

Referring back to FIG. 4, a lysed sample prepared by lysing module 160 is received by mixing module 166. Mixing module 166 includes a mixing zone 958. In this zone, the lysed cell sample is contacted, such as by mixing, with an amount of reagent received from the reagent source module 152. 40 Reagent source module 152 includes a reagent microdroplet preparation zone (RMPZ) 434, which preferably operates to prepare a microdroplet having a predetermined volume of reagent

#### a. Reagent Input Module

Reagent input module **152** is essentially the same as microdroplet formation module **158**, however, it is specifically designed for formation of a microdroplet of reagent having a predetermined volume which will yield a desired ratio of reagent to sample when mixed with the microdroplet from 50 cell lysing module **160**. Module **152** includes an input port **420**, a valve **422**, and an actuator **172**, each of which joins a reagent source channel **428**. An overflow channel **424**, which also joins reagents source channel **428**, may also be provided. Actuator **172** may include a second positioning element **432** 55 to prevent liquid from entering therein.

Reagent materials, which preferably comprise at least one liquid, are introduced via input port **420**, such as with a pipette or syringe. Examples of suitable reagent materials include substances to facilitate further processing of the lysed cell 60 sample, such as enzymes and other materials for amplifying DNA therein by polymerase chain reaction (PCR). The reagent material moves downstream within reagent source channel **428** until a downstream portion of the reagent material contacts a positioning element **426**. Any additional 65 reagent material that continues to be received within reagent source module preferably enters overflow channel **424**. When

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the introduction of reagent is complete, valve 422 is closed to prevent reagent from exiting reagent source channel via reagent source port 420.

#### b. Mixing Module

Mixing zone 958 of the mixing module includes adjoined first and second channels 410, 412. Materials moving downstream toward mixing zone 958 contact one another and preferably mix therein. Because of the micro-scale dimensions of mixing zone 958, the sample and reagent materials preferably mix by diffusion even in the absence of other sources of mass transport, such as mechanical agitation. It should be understood however, that agitation forces, such as acoustic waves may be applied to enhance mixing within mixing zone 958. c. Operation of Mixing Module and Reagent Input Module

Reagent source module 152 and mixing module 166 preferably operate as follows. When a lysed sample from lysing zone 950 is ready to be mixed with reagent material, actuator 172 is actuated to prepare a microdroplet of reagent. The microdroplet of reagent is prepared from the portion of reagent material downstream of an opening 430 of actuator 172 and upstream of positioning element 427. Thus, assuming that the dimensions of the reagent source channel 428 are constant, the volume of the microdroplet of reagent is determined by the distance between the positioning element 426 and the actuator opening 430.

The microdroplet of reagent moves downstream toward channel 412 of reagent mixing zone. Meanwhile, a sample of lysed material, such as a lysed microdroplet, is moved downstream from lysing zone 950 toward channel 410 of mixing zone 958. Actuator 170 may provide the motive force to move the lysed microdroplet downstream. Alternatively, as discussed above, another actuator may be disposed upstream of lysing zone 950 but downstream of actuator 170 to provide the necessary motive force.

The sample and reagent material enter a downstream channel 438 of mixing zone 958, where the materials contact and mix. Because both the lysed sample and reagent material are mixed in the form of microdroplets, mixing zone 958 prepares an amount of mixed material having a predetermined ratio of sample to reagent. The volumes of microdroplets prepared within microfluidic device 110 are preferably independent of physical properties, such as viscosity, electrical conductivity, and osmotic strength, of the microdroplets. Thus, mixing zone 958 prepares an amount of mixed material having a sample to reagent material that is also independent of the physical and chemical properties of the mixed materials. A vent 440, which is downstream of the various zones of the microfluidic device 110 ensures that downstream pressure buildup does not inhibit downstream movement of samples within microfluidic device 110.

#### 5. DNA Manipulation Module

The mixed lysed cell sample and reagent are received within a DNA manipulation zone 971 of DNA manipulation module 162. Module 162 can perform, for example, restriction, digestion, ligation, hybridization and amplification of DNA material. In one embodiment, DNA manipulation zone 971 is configured to perform PCR amplification of nucleic acids present within the lysed cell sample. Vent 440 prevents pressure from increasing within zone 971 as the lysed cell sample and reagent are being introduced thereto. Valves 972 and 973 of DNA manipulation module 162 may be closed to prevent substances therein zone from exiting, such as by evaporation, during PCR amplification. The DNA manipulation zone is configured with heat sources under control of computer 127 to allow thermal cycling of DNA manipulation zone during amplification, as understood by one of skill in the

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System 901 includes also includes a detector 981 to detect the presence of amplified polynucleotides produced by PCR. Detector 981 is preferably an optical detector in optical communication, such as by a fiber optic 981, with zone 971. A light source, such as a laser diode, introduces light to DNA Manipulation zone 971 to generate fluorescence indicative of the amount of amplified polynucleotides present therein. The fluorescence arises from fluorescent tags, included in the reagent and associated with the polynucleotides upon ampli-

#### C. Preferred Positioning Elements

Preferred positioning elements are discussed below.

#### 1. Non-Wetting Positioning Elements

A positioning element 979 may be formed by a non-wetting material disposed to contact a microfluidic sample. The physio-chemical properties of the non-wetting material are chosen upon considering the type of liquid forming the microfluidic sample. For example, where the microfluidic sample is an aqueous sample, the positioning element pref- 20 erably comprises a hydrophobic material. An exemplary hydrophobic material includes a non-polar organic compound, such as an aliphatic silane, which can be formed by modifying an internal surface of microfluidic device 901. For microfluidic samples formed of organic solvents, the non- 25 wetting material may comprise a hydrophilic material.

When microfluidic sample 808 encounters positioning element 979, the liquid of the microfluidic sample experiences an increased surface tension at downstream interface 810, which increased surface tension inhibits continued downstream motion of microfluidic sample 808. Increasing the gas pressure difference between upstream and downstream portions of the microfluidic sample overcomes the resistance and moves the microfluidic sample downstream.

#### 2. Capillary Assisted Positioning Elements

Referring to FIGS. 10a-10c, another type of positioning element may be formed by modifying the dimensions of the microfluidic channel to form a capillary assisted positioning element (CAFB) 700. A CAFB comprises an upstream feed 40 the opening of vent 508, the upstream gas dissipates through zone 702, a loading zone 704, and a stop zone 704. A microfluidic sample 720 encountering the CAFB moves downstream until a downstream interface 710 of the microfluidic sample contacts upstream surfaces 714 of the loading zone **706.** At this point, capillary action causes the microfluidic 45 sample to move downstream until the downstream sample interface 710 encounters the opening 712 between the loading zone 704 and the stop zone 706. Surface tension resists the tendency of the microfluidic sample to continue downstream past opening 714. Thus, the microfluidic sample 720 is posi- 50 tioned at a predetermined location along the channel axis with respect to positioning element 700.

The volume of the microfluidic sample encountering the CAFB preferably has a larger volume than a volume of the loading zone 704 to ensure that the microfluidic sample will 55 advance fully to opening. For fluids that have similar surface tensions and interface properties as water, the depth d.sub.1 of the loading zone 704 is preferably about 50% or less of the respective depths d.sub.2, d.sub.3 of the stop and feed zones.

The tendency of a microfluidic sample to move in a given 60 direction is governed by the ratio between the mean radius of curvature (MRC) of the front of the microfluidic sample and the MRC of the back of the microfluidic sample. These curvatures depend upon the contact angle of the fluid of the sample and the dimensions of the zone in which the microdroplet is moving. A MRC r.sub.1 of a microdroplet interface in the loading zone is preferably smaller than a MRC r.sub.2

of a droplet interface within the feed zone or a MRC r.sub.3 of a droplet interface within the stop zone. The MRC r.sub.2 is preferably larger than the MRC r.sub.3. Thus, the radius of curvature of the downstream microdroplet interface increases upon encountering the stop zone thereby inhibiting further downstream movement. Preferably, the contact angle of the fluid with the wall is substantially constant throughout the capillary assisted loading zone.

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#### 3. Vented Positioning Elements

Referring to FIGS. 11a-11c, a positioning element 500 operates to position a microfluidic sample 502 by reducing the gas pressure acting upon an upstream portion 504 of the microfluidic sample relative to the gas pressure acting upon a downstream portion 506 of the microfluidic sample. Positioning element 500 includes a vent 508 disposed in gaseous communication with a zone 510 along which microfluidic sample 502 moves. Vent 508 preferably communicates with zone 510 via a passage 526. The zone may be for example, a channel or conduit. Positioning element 500 may also include a second positioning element 516, such as a non-wetting material, to substantially prevent fluid from the microfluidic sample from contacting the vent.

An open state of a valve 512 allows passage of gas between zone 510 and vent 508. A closed state of valve 512 prevents such passage of gas. Valve 514 is preferably thermally actuated and includes a mass 514 of TRS.

An actuator 518 is disposed upstream of positioning element 500. Actuator 518 is preferably a gas actuator and may include a heat source 520 to heat a gas associated with actuator 518. Actuator 518 may include a positioning element 522, such as non-wetting material, to substantially prevent fluid from the microfluidic sample from entering therein.

Positioning element 500 preferably operates as follows. Referring to FIG. 11a, microfluidic sample 502 moves down-35 stream in the direction of arrow 524. Microfluidic sample is preferably moved by a gas pressure provided from an upstream actuator, which is not shown in FIGS. 9a-9c. The gas pressure acts upon upstream portion 504.

Referring to FIG. 11b, when upstream portion 504 passes vent **508**, thereby reducing the upstream pressure. The pressure reduction, which preferably equalizes the downstream and upstream pressures, reduces or eliminates the motive force tending to urge the microfluidic sample downstream.

Referring to FIG. 11c, valve 512 is closed to prevent passage of gas between zone 510 and vent 508. Preferably, TRS 514 moves into passage 526. Upon closing valve 512, the actuation of actuator 518 provides a motive force to move microfluidic sample 502 downstream in the direction of arrow **528** for further processing.

#### 4. Active Fluid Positioning Elements

Referring to FIGS. 15a-15c, a microdroplet preparation module 652 has a microdroplet preparation zone 650, an active fluid positioning element 654, an actuator 656, and a valve 658. A second actuator 660 is operatively associated with the active positioning element 654 to introduce a microfluidic sample 666 to the microdroplet preparation zone 650. Second actuator 660 is preferably located upstream from valve 658. Microdroplet preparation module 652 prepares a microdroplet 668, which has a predetermined volume from the microfluidic sample 666 received therein.

In operation, microfluidic preparation module 652 receives the microfluidic sample 666, which moves downstream because of a motive force provided by the second actuator **660**. The motive force is preferably an upstream gas pressure, which is greater than a downstream gas pressure acting upon the microfluidic sample 666. The microfluidic sample moves

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downstream until a downstream portion **670** thereof encounters active positioning element **654**, which preferably comprises a sensor **672** having electrical leads **674**. The leads **674** are in electrical communication with I/O pins of the microfluidic device to allow signals from sensor **672** to be received by a DAQ.

Sensing element 672 is preferably a pair of electrical contacts. To sense the presence of the liquid, DAQ 126 applies a small voltage across leads 674 and measures the resultant current. As the liquid of the microfluidic sample contacts the first and second contacts, the current passing therebetween changes, thereby indicating to DAQ 126 that the liquid has arrived at sensor 672.

Upon recognition that the liquid has arrived at sensor **672**, the DAQ instructs second actuator **660** to decrease a downstream motive force acting upon the microfluidic sample **666**. For example, DAQ may reduce a current flowing through a heat source **676** associated with second actuator **660** thereby reducing a temperature of a gas therein. The temperature reduction reduces the gas pressure acting upon a upstream portion **678** of microfluidic sample thereby inhibiting the downstream motion of the microfluidic sample **666**. The microfluidic sample is positioned such that a first portion **680** is located downstream of actuator **656** and a second portion <sup>25</sup> **682** is located upstream of actuator **656**.

To prepare microdroplet **668**, DAQ **126** actuates actuator to provide a motive force which prepares the microdroplet **668** from the first portion **680** of microfluidic sample **666**. Microdroplet **668** moves downstream while the second portion **682** of the microfluidic sample **666** moves upstream from actuator **656**. During microdroplet preparation, valve **658** may be closed to substantially isolate the actuator **656** from second actuator **660** and other upstream portions of the microfluidic device

The active positioning element preferably operates as a closed loop element that provides feedback from sensor **672** to the DAQ. The feedback is indicated when a microfluidic sample has reached a predetermined position within the 40 microfluidic device. Upon receiving the feedback, the DAQ changes the state of the actuator providing the motive force to move the microdroplet.

While the above invention has been described with reference to certain preferred embodiments, it should be kept in 45 mind that the scope of the present invention is not limited to these. Thus, one skilled in the art may find variations of these preferred embodiments which, nevertheless, fall within the spirit of the present invention, whose scope is defined by the claims set forth below.

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What is claimed is:

1. A method of amplifying a nucleic acid-containing sample within a microfluidic device, the method comprising: moving the sample from an upstream channel of the microfluidic device into a DNA manipulation module located downstream of the upstream channel, the DNA manipulation module including a DNA manipulation zone configured to perform amplification of the sample, a first valve disposed upstream of the DNA manipulation zone, and a second valve disposed downstream of the DNA manipulation zone, the only ingress to and egress from the DNA manipulation zone being through the first valve and the second valve;

receiving the sample in the DNA manipulation zone;

closing the first valve and the second valve such that gas and liquid are prevented from flowing into or out of the DNA manipulation zone; and

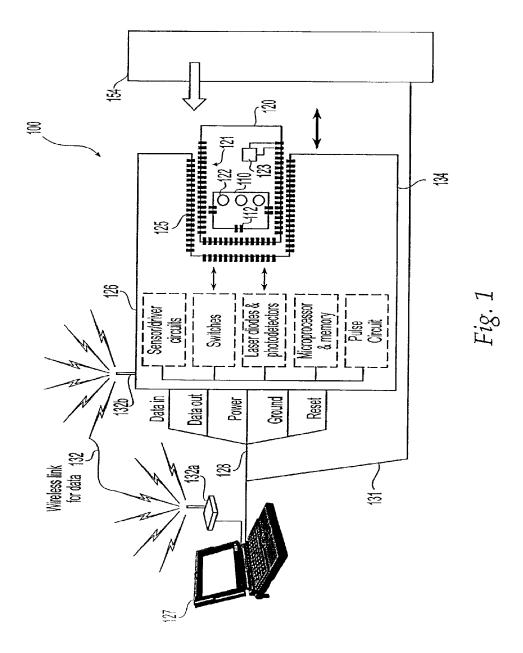
thermal cycling the sample in the DNA manipulation zone.

- 2. The method of claim 1, wherein moving the sample from an upstream channel into the DNA manipulation module comprises actuating a gas actuator.
- 3. The method of claim 2, wherein actuating the gas actuator comprises increasing a gas pressure within the upstream channel relative to a gas pressure within the DNA manipulation zone.
- 4. The method of claim 2, wherein actuating the gas actuator comprises decreasing a gas pressure within the DNA manipulation zone relative to a gas pressure within the upstream channel.
- 5. The method of claim 1, wherein closing the first valve and the second valve comprises beating a thermally responsive substance in the first valve and the second valve.
- **6**. The method of claim **1**, wherein thermal cycling the sample in the DNA manipulation zone comprises cyclically heating the sample with a computer-controlled heat source in thermal contact with the DNA manipulation zone.
- 7. The method of claim 1, wherein thermal cycling the sample in the DNA manipulation zone comprises controlling a plurality of resistive heaters in thermal contact with the DNA manipulation zone.
- **8**. The method of claim **1**, further comprising identifying the presence of one or more nucleic acids within the DNA manipulation zone.
- **9.** The method of claim **8**, wherein identifying the presence of one or more nucleic acids comprises introducing light into the DNA manipulation zone, the light selected to generate fluorescence indicative of the presence of amplified nucleic acids within the DNA manipulation zone.
- 10. The method of claim 1, further comprising identifying an amount of one or more amplified nucleic acids within the DNA manipulation zone.

\* \* \* \* \*

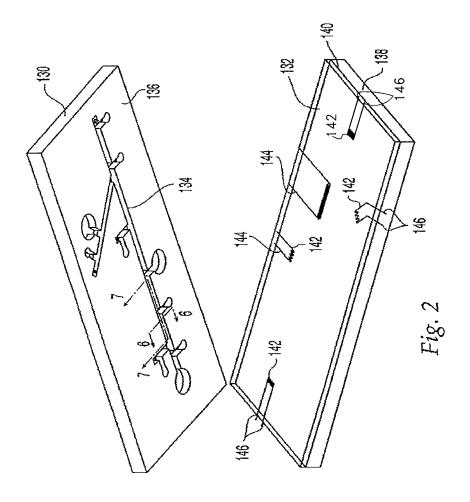
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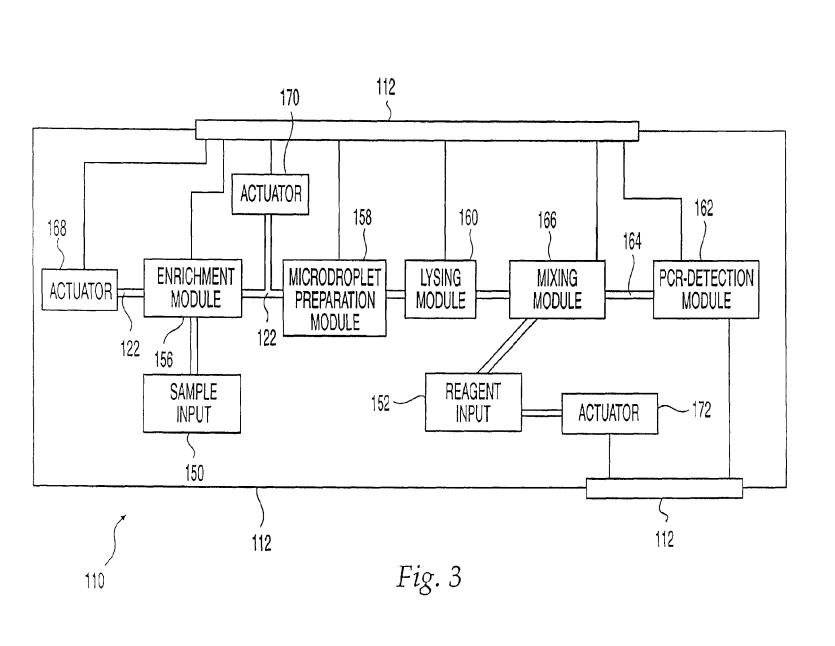
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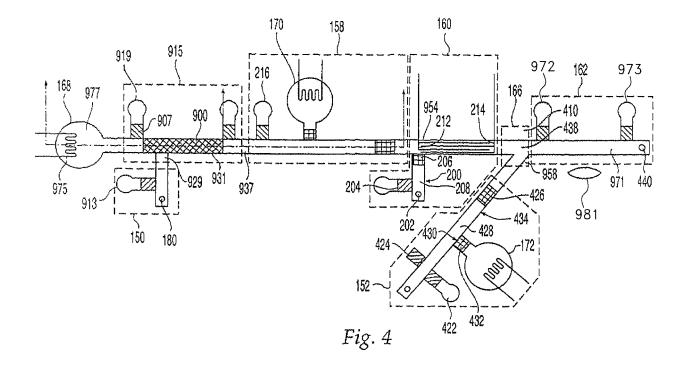
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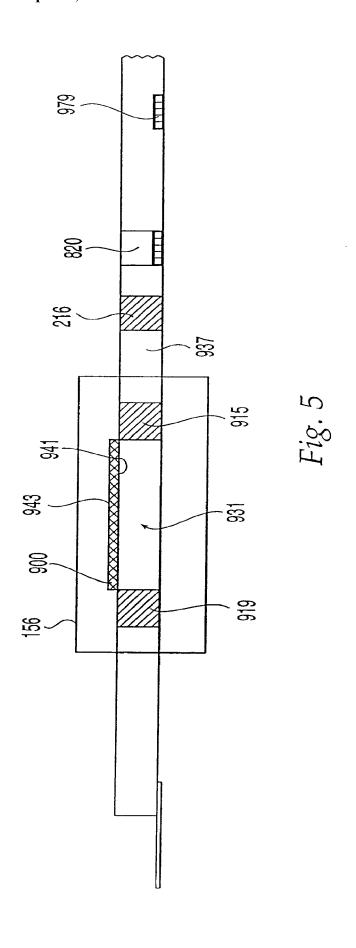
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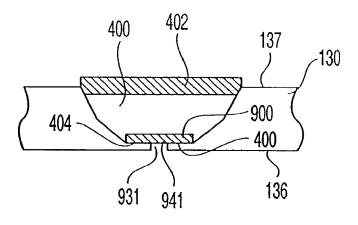


Fig. 6

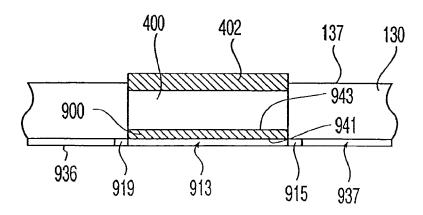


Fig. 7

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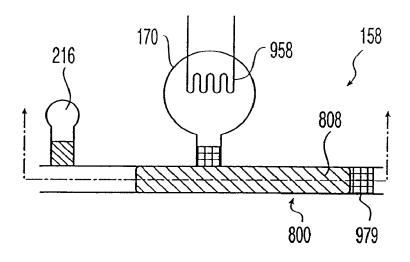


Fig. 8a

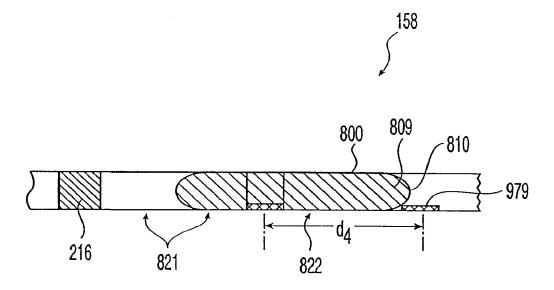


Fig. 8b

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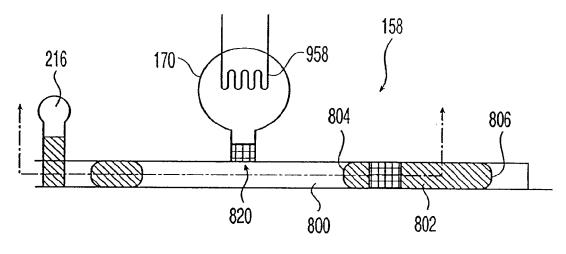


Fig. 9a

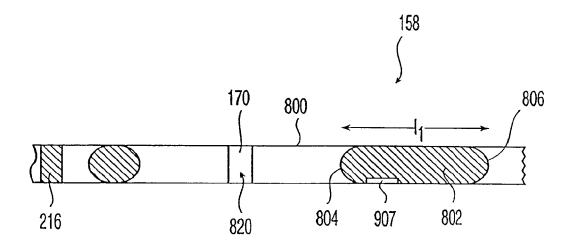
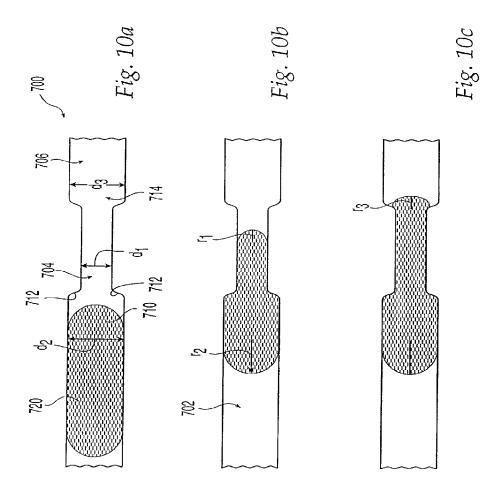


Fig. 9b

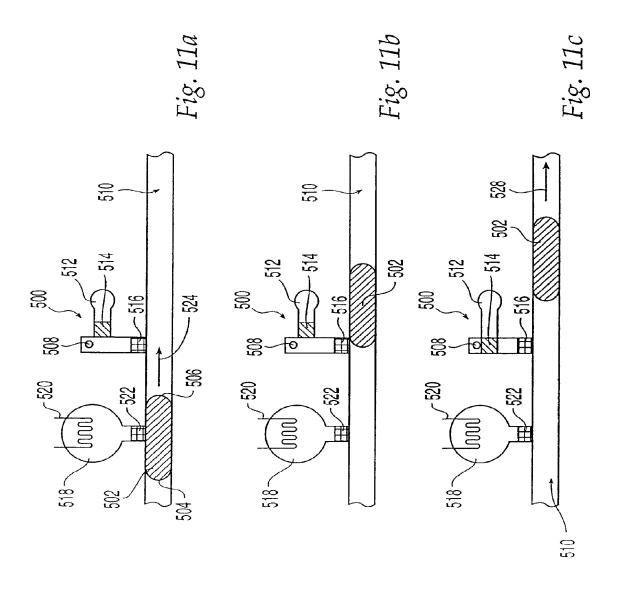
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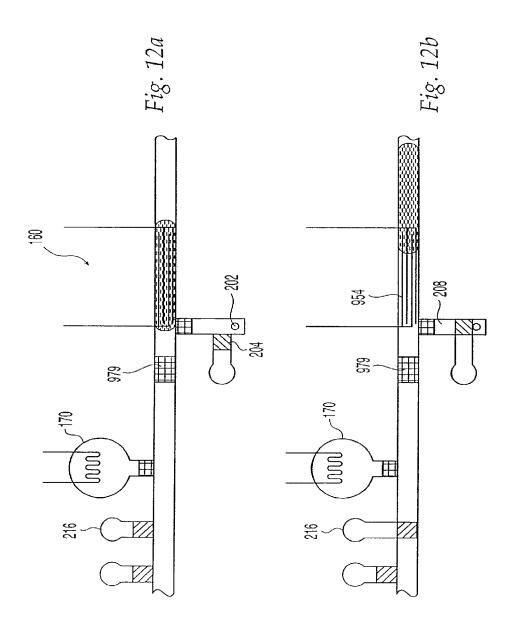
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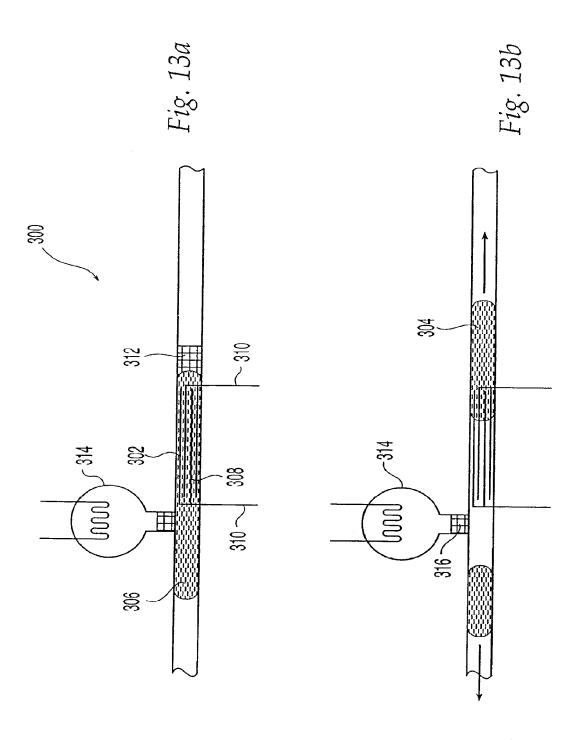
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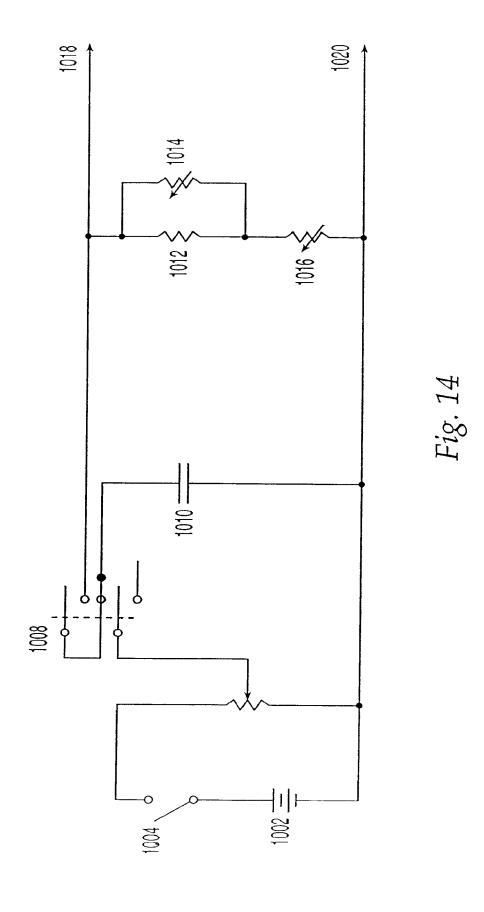
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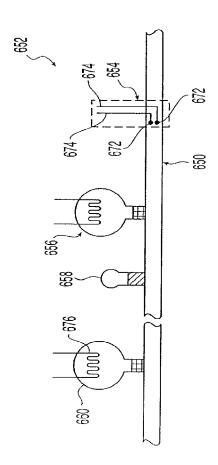


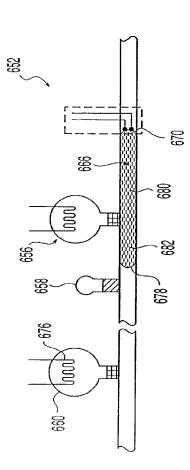
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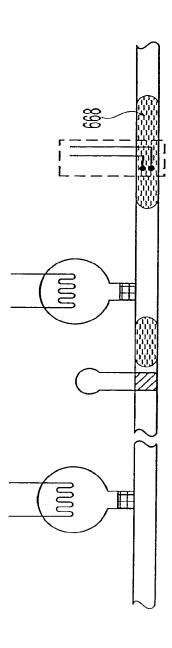


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US 8,703,069 B2

Fig. 15c



#### UNITED STATES PATENT AND TRADEMARK OFFICE

## CERTIFICATE OF CORRECTION

PATENT NO. : 8,703,069 B2

APPLICATION NO. : 13/620452

DATED : April 22, 2014

INVENTOR(S) : Handique et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title Page

Page 1 (item 57, Abstract) at line 13, Change "that as" to --that gas--.

Page 8 (item 56, Other Publications) at column 2, line 2, change "Polyamidomine" to --Polyamidoamine--.

In the Specification

In column 2 at line 23, change "actuatators" to --actuators--.

In column 2 at line 36, change "device." to --device;--.

In column 2 at line 38, change "FIG." to --FIG. 1;--.

In column 4 at lines 43-44, change "photo-lithographic" to --photolithographic--.

In column 4 at line 45, change "penult" to --permit--.

In column 5, line 67 to column 6, line 1, change "actuatators" to --actuators--.

In column 6 at line 14, change "9.sctn.01" to --901--.

In column 6 at line 30, change "down stream" to --downstream--.

In column 9 at line 44, change "length 1.sub.1" to --length 1.sub.1--.

In column 13 at line 1, after "901" delete "includes".

Signed and Sealed this Second Day of December, 2014

Michelle K. Lee

Michelle K. Lee

Deputy Director of the United States Patent and Trademark Office

# EXHIBIT 3



# (12) United States Patent

Handique et al.

#### US 7,998,708 B2 (10) **Patent No.:**

(45) **Date of Patent:** Aug. 16, 2011

#### (54) MICROFLUIDIC SYSTEM FOR AMPLIFYING AND DETECTING POLYNUCLEOTIDES IN **PARALLEL**

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MI (US)

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(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

(21) Appl. No.: 11/985,577

(22) Filed: Nov. 14, 2007

#### (65)**Prior Publication Data**

US 2008/0182301 A1 Jul. 31, 2008

#### Related U.S. Application Data

- (63) Continuation-in-part of application No. 11/728,964, filed on Mar. 26, 2007.
- (60) Provisional application No. 60/859,284, filed on Nov. 14, 2006, provisional application No. 60/959,437, filed on Jul. 13, 2007, provisional application No. 60/786,007, filed on Mar. 24, 2006.
- (51) Int. Cl. C12Q 1/68 (2006.01)C12P 19/34 (2006.01)
- (58) Field of Classification Search ...... None See application file for complete search history.

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Primary Examiner — Young J Kim

for PCT/US07/07513.

(74) Attorney, Agent, or Firm — Knobbe Martens Olson & Bear LLP

#### (57)ABSTRACT

The present technology provides for an apparatus for detecting polynucleotides in samples, particularly from biological samples. The technology more particularly relates to microfluidic systems that carry out PCR on nucleotides of interest within microfluidic channels, and detect those nucleotides. The apparatus includes a microfluidic cartridge that is configured to accept a plurality of samples, and which can carry out PCR on each sample individually, or a group of, or all of the plurality of samples simultaneously.

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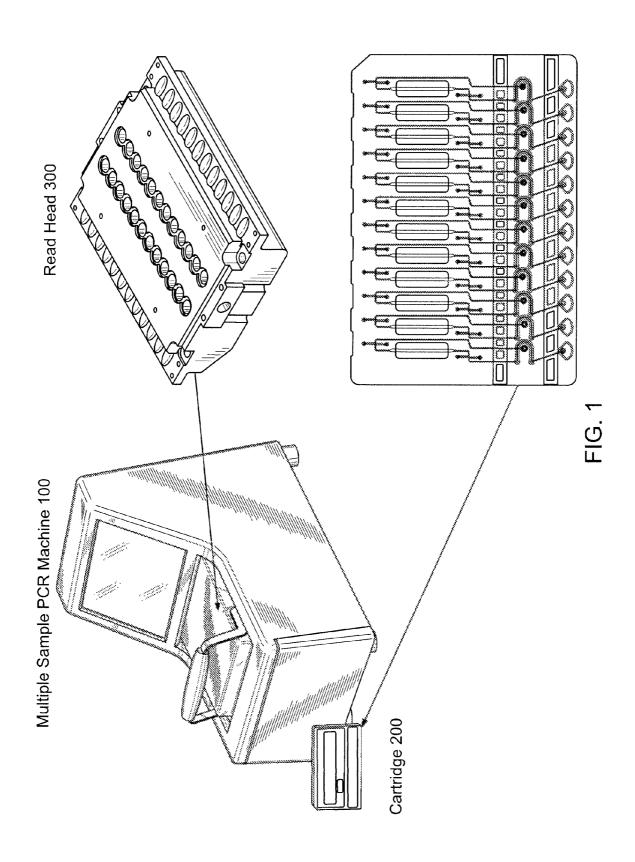
Page 2

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## Sample Preparation Kit

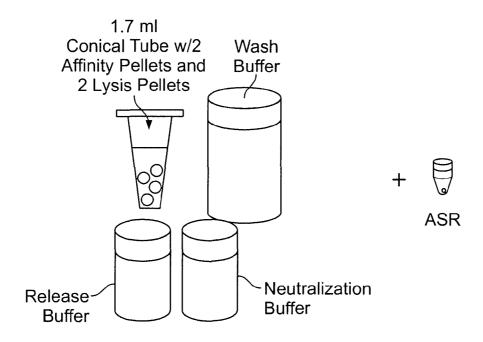


FIG. 2

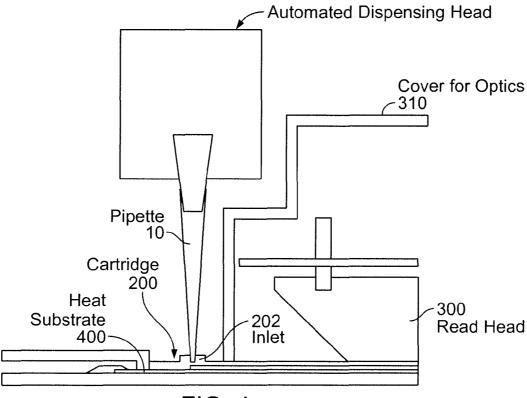


FIG. 4

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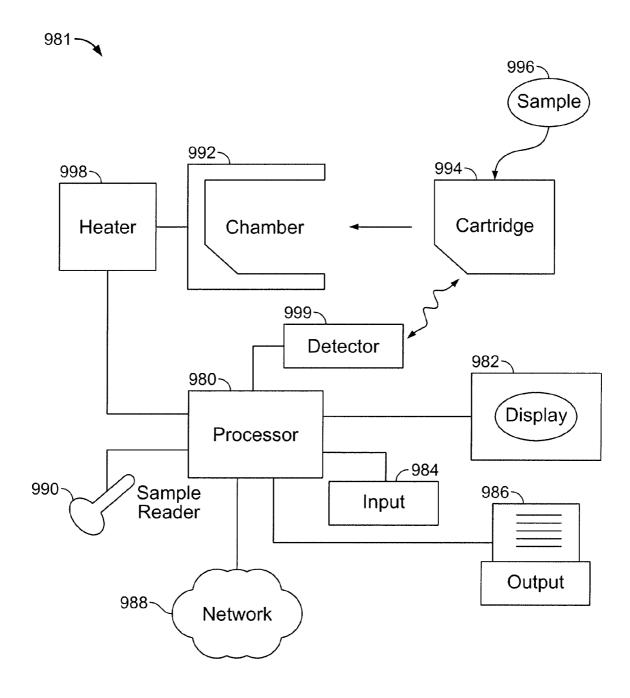


FIG. 3

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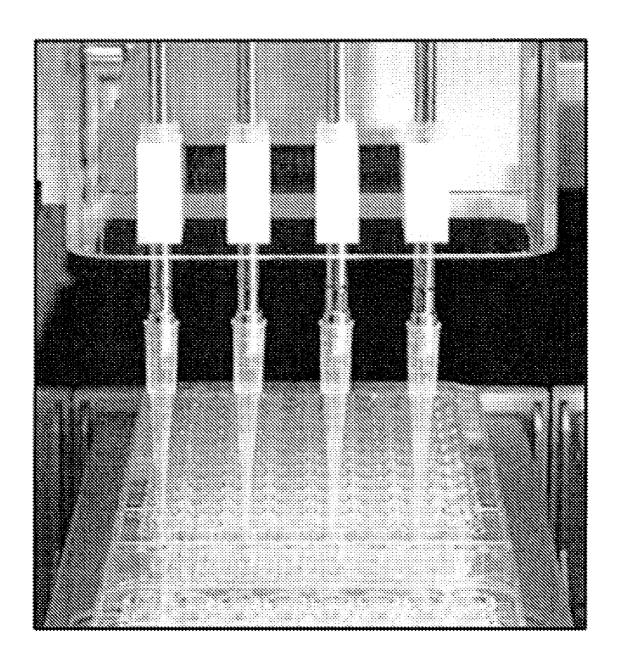


FIG. 5

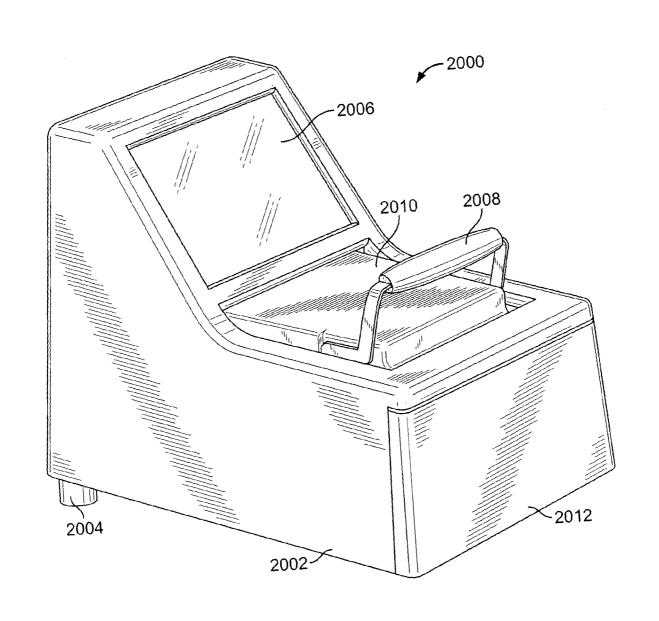


FIG. 6A

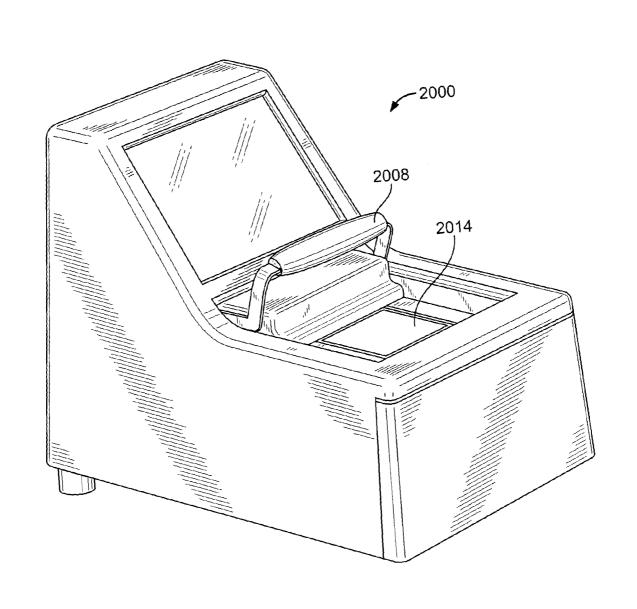


FIG. 6B

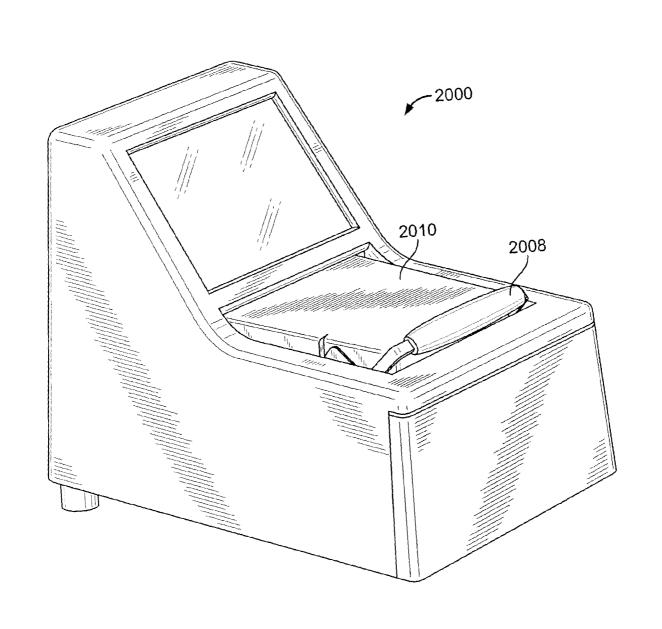
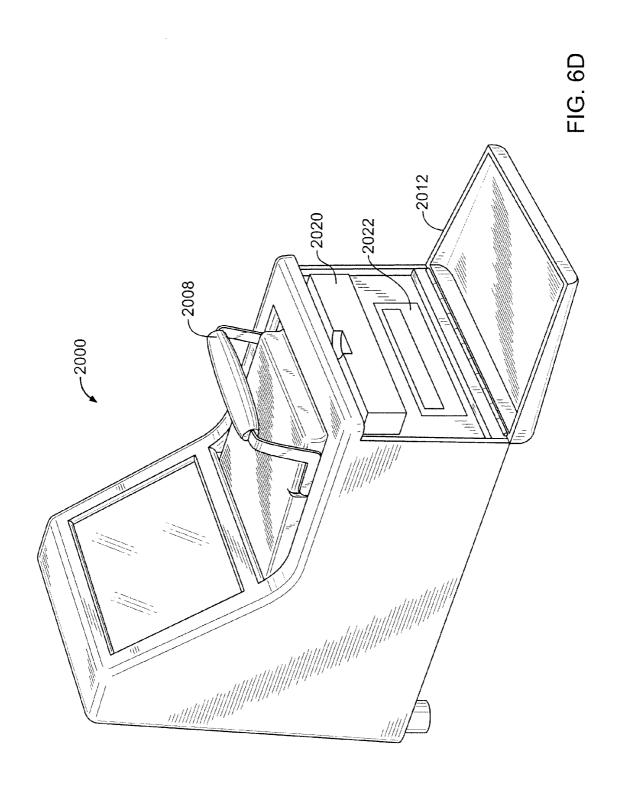


FIG. 6C

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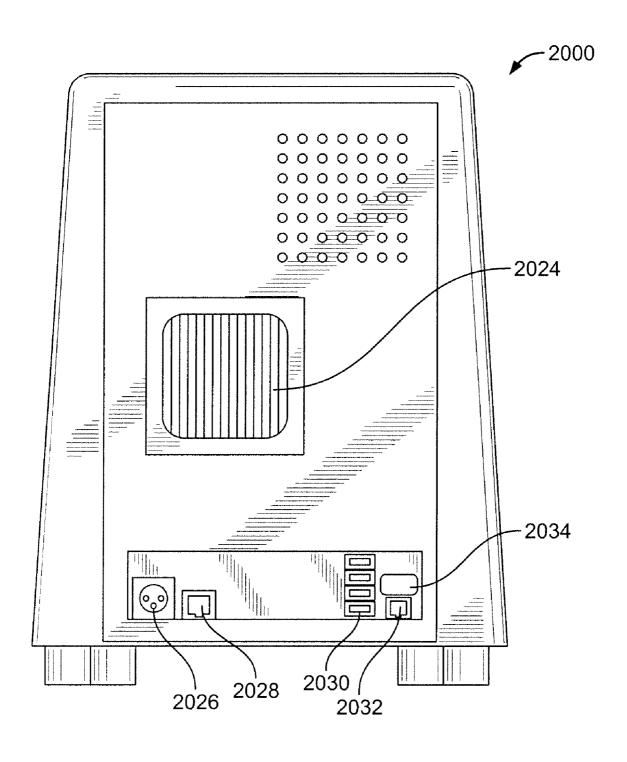


FIG. 6E

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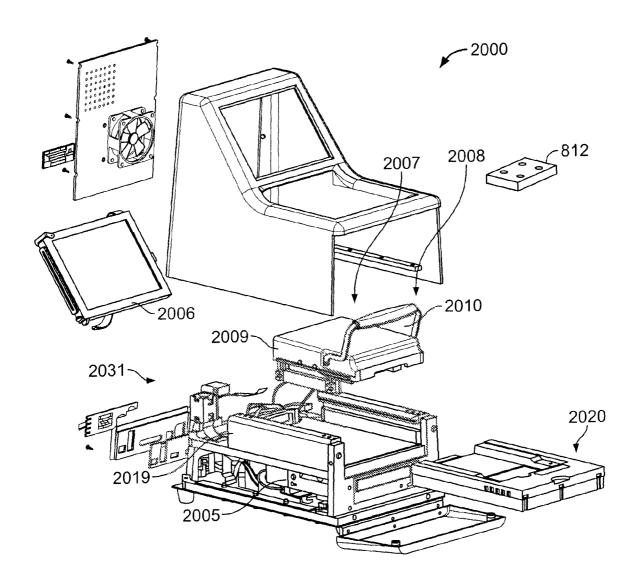


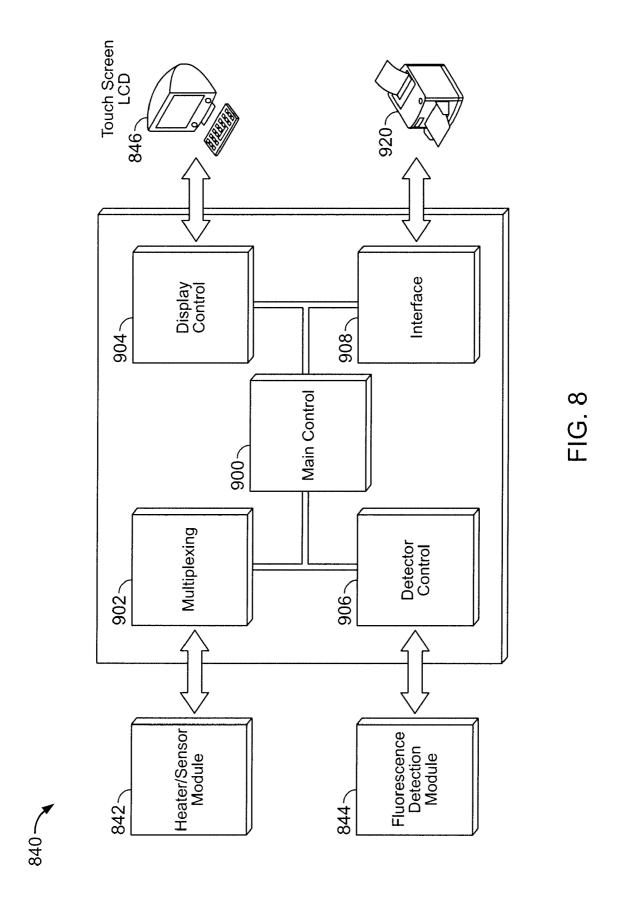
FIG. 7

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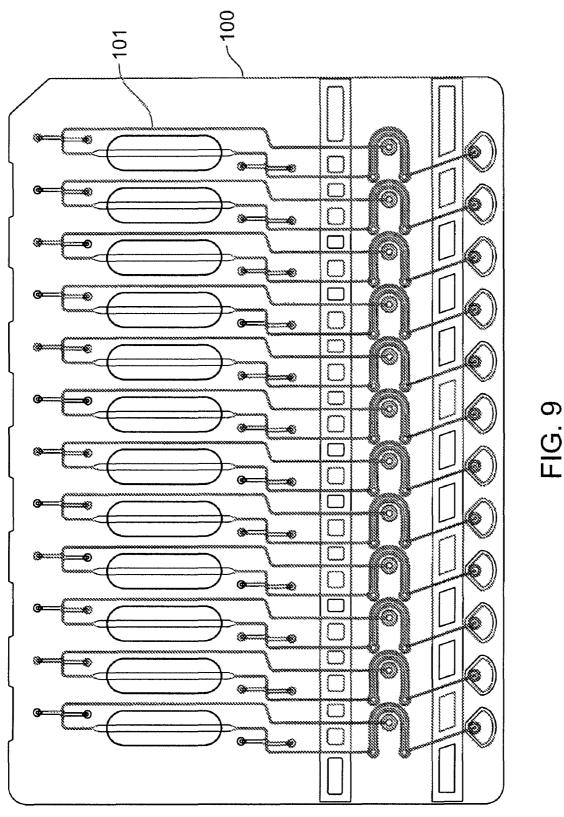
**Sheet 11 of 61** 

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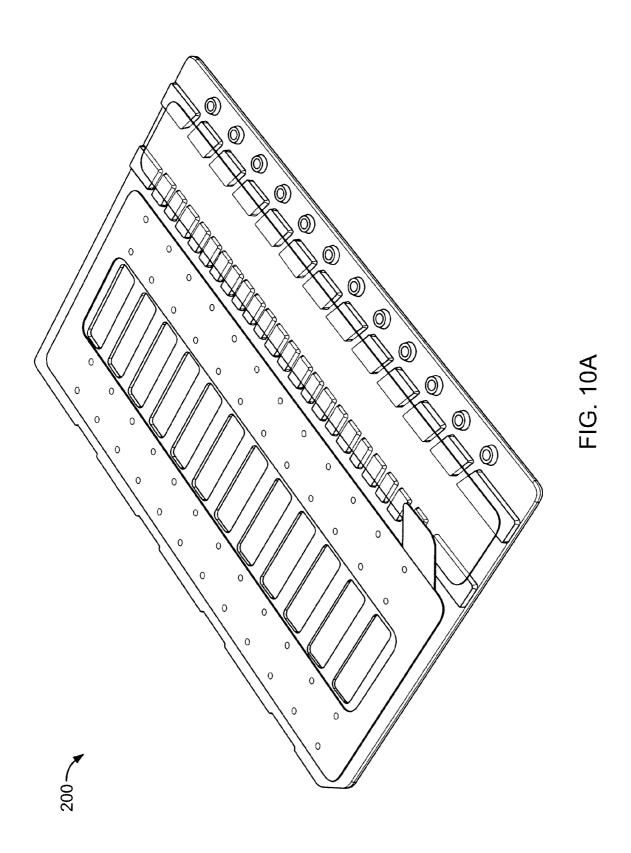
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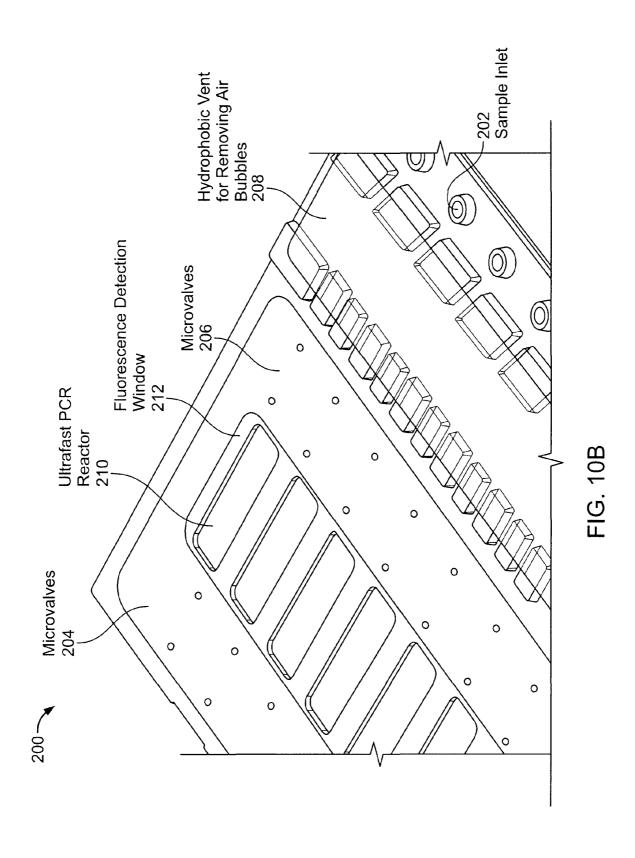
Aug. 16, 2011

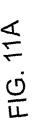
**Sheet 13 of 61** 

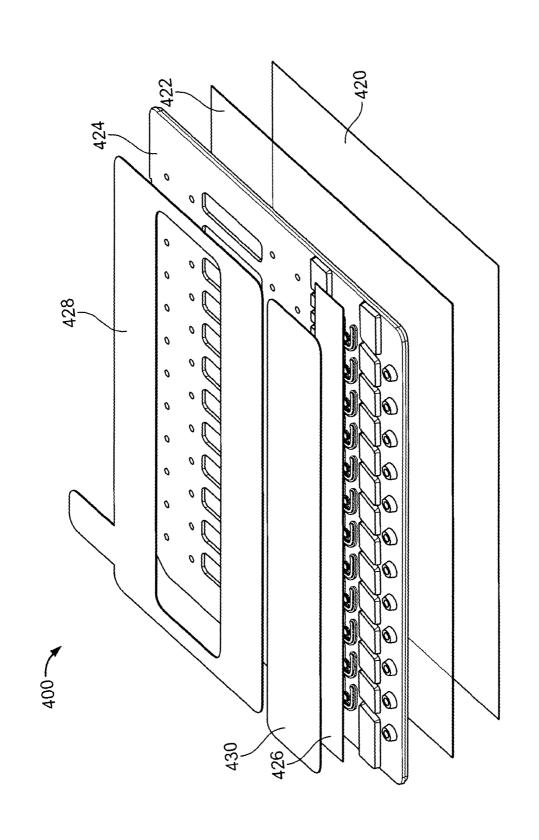


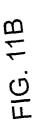
Aug. 16, 2011

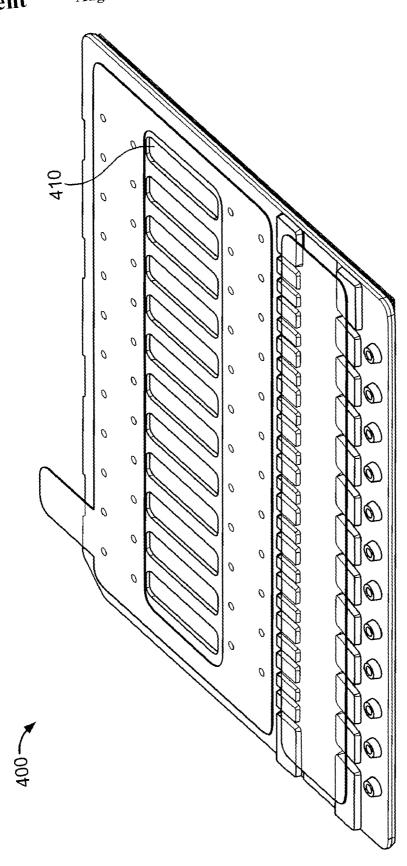
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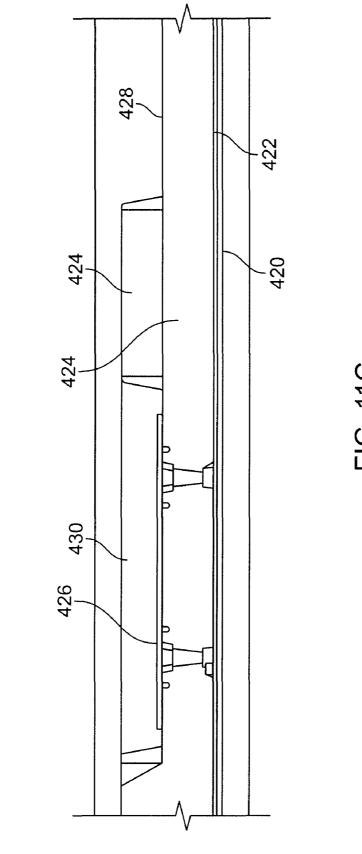
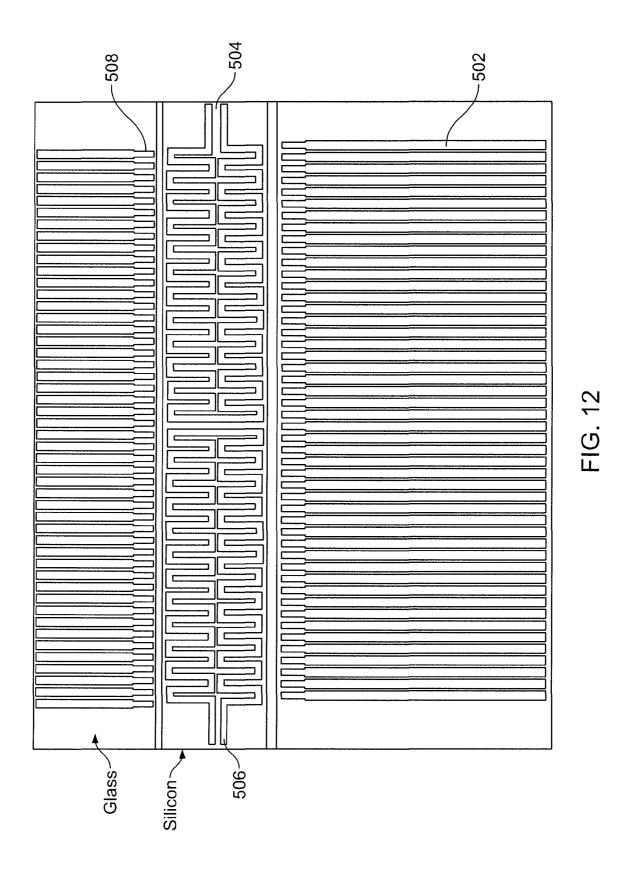


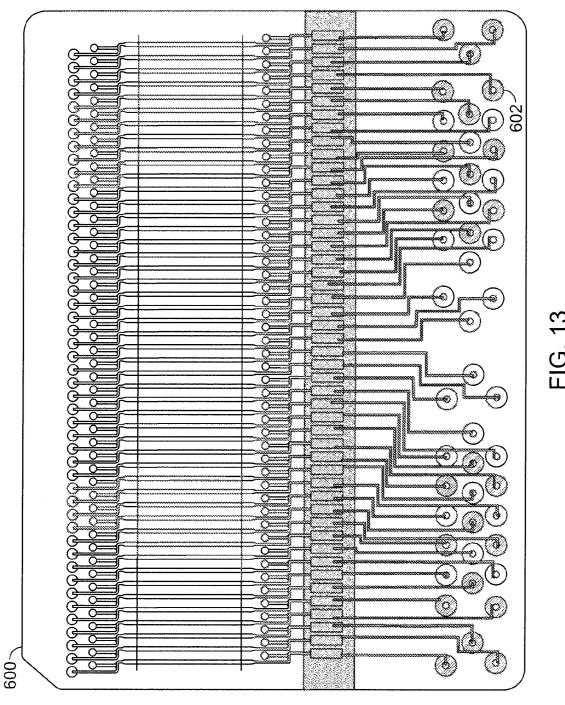
FIG. 11C

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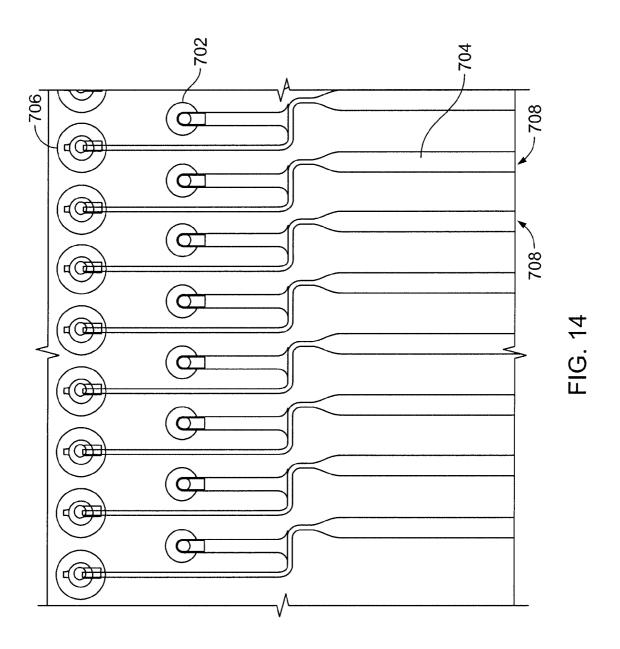
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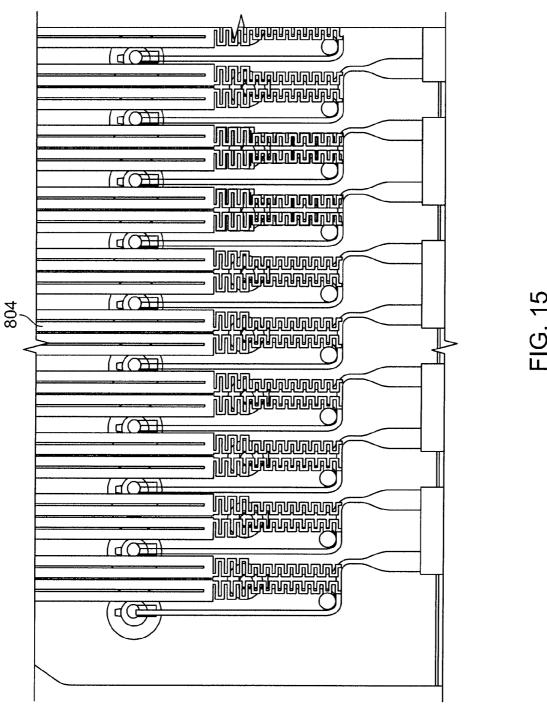
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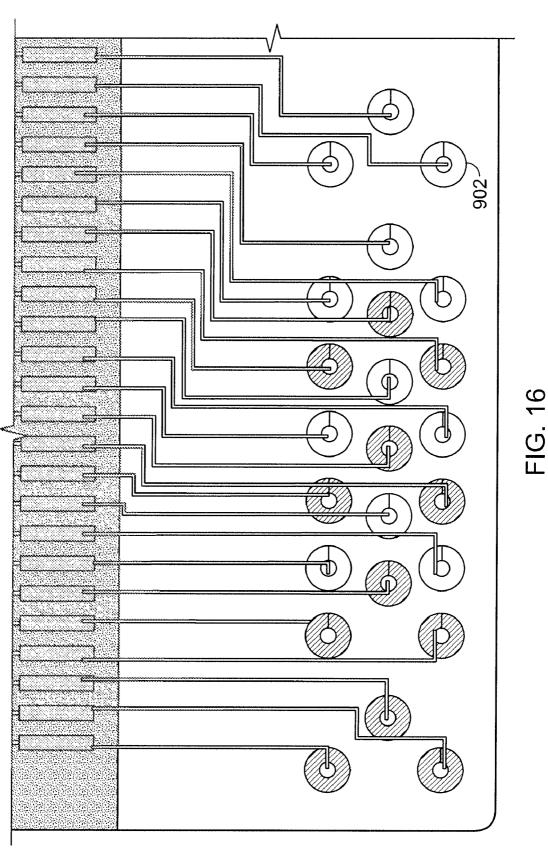
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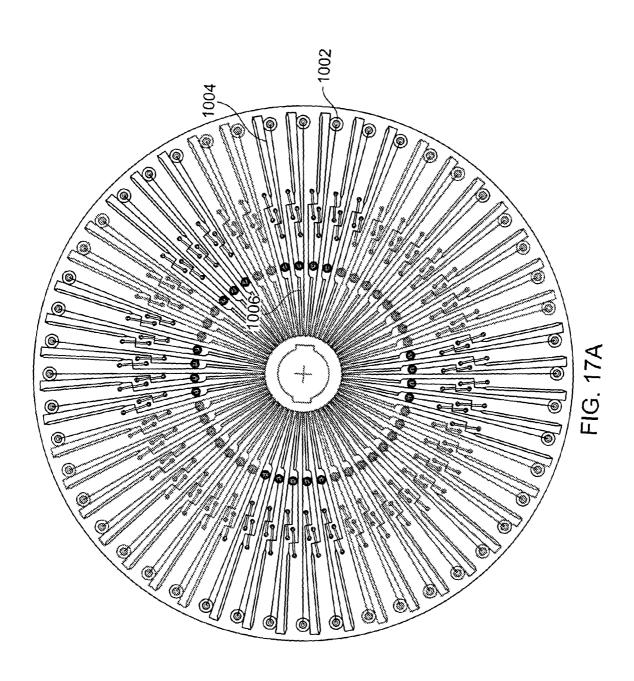
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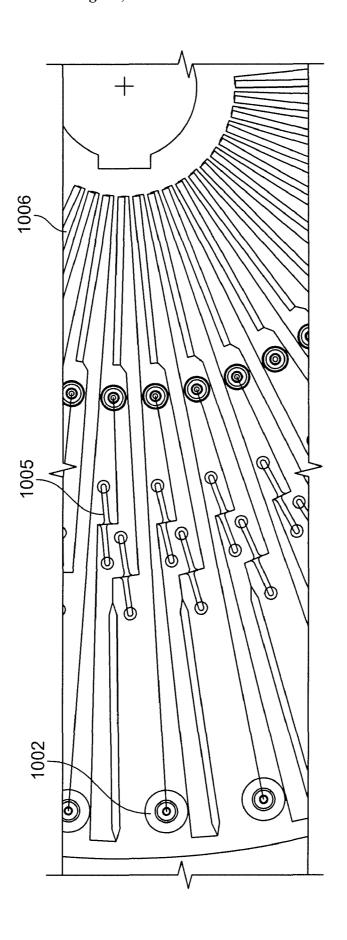
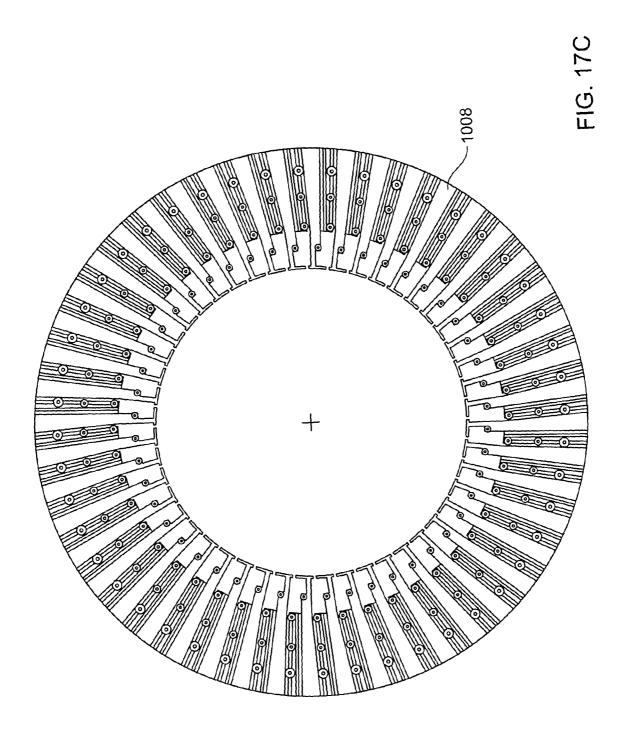


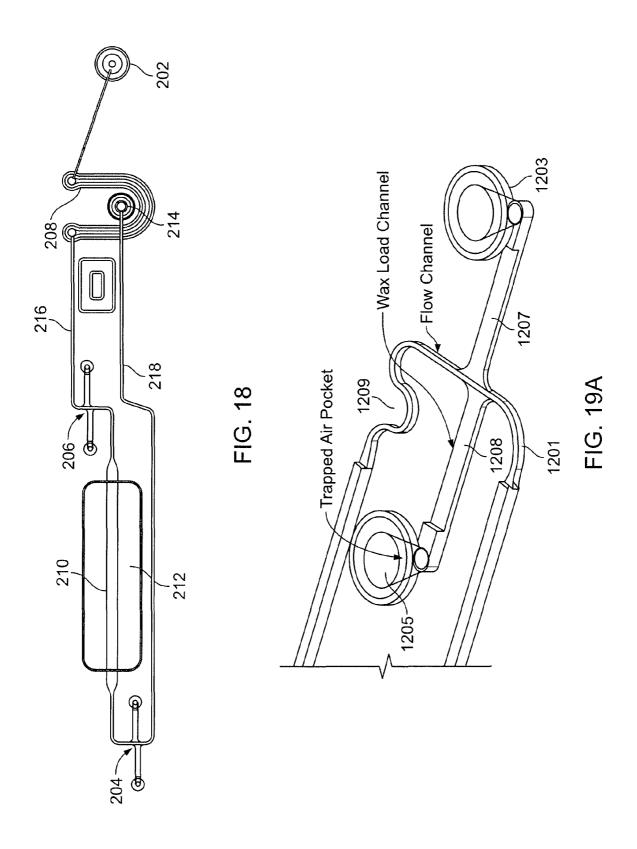
FIG. 17B

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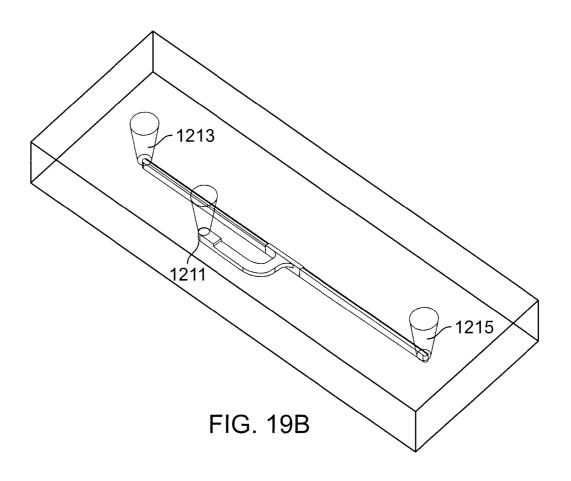


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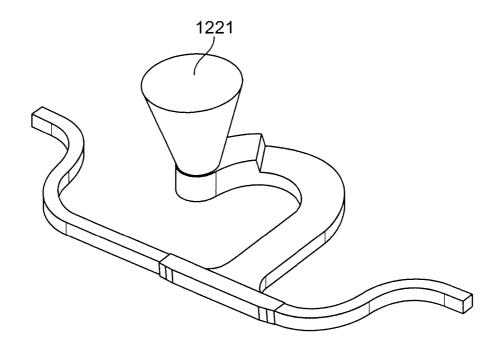
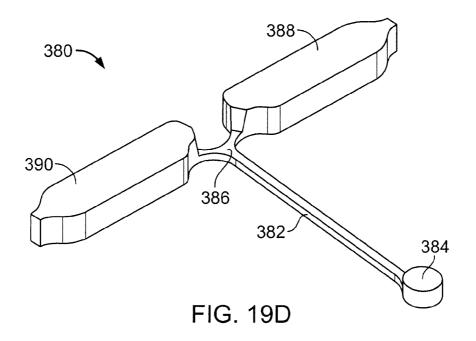


FIG. 19C

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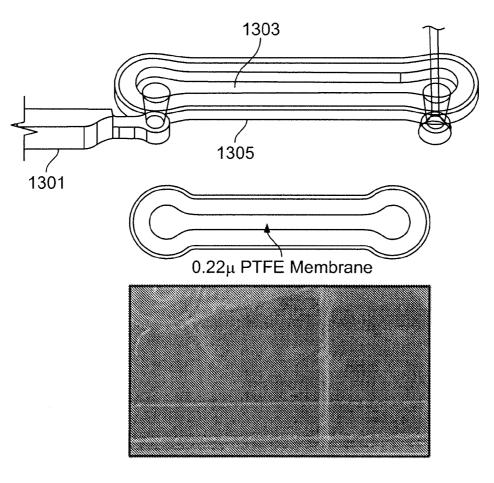
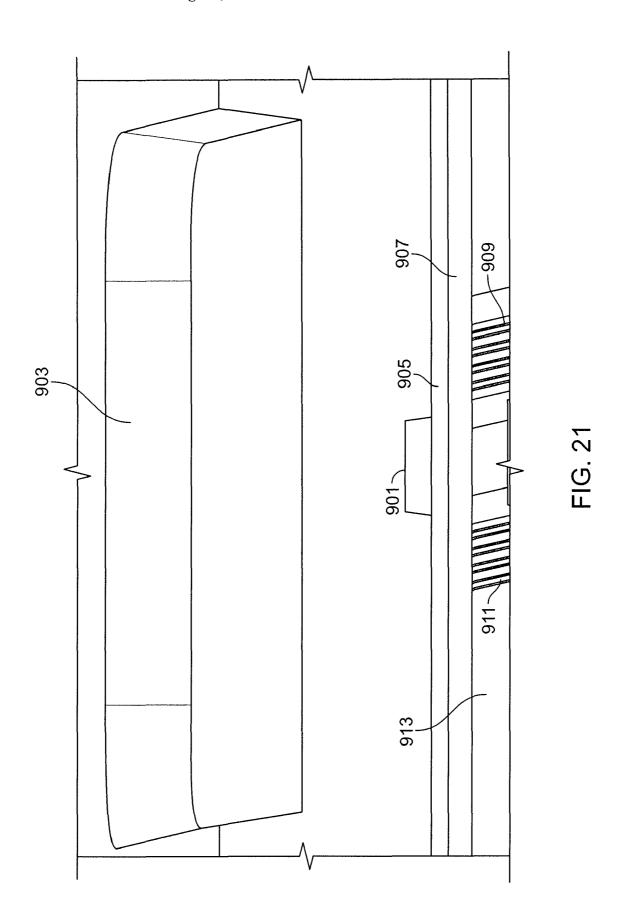
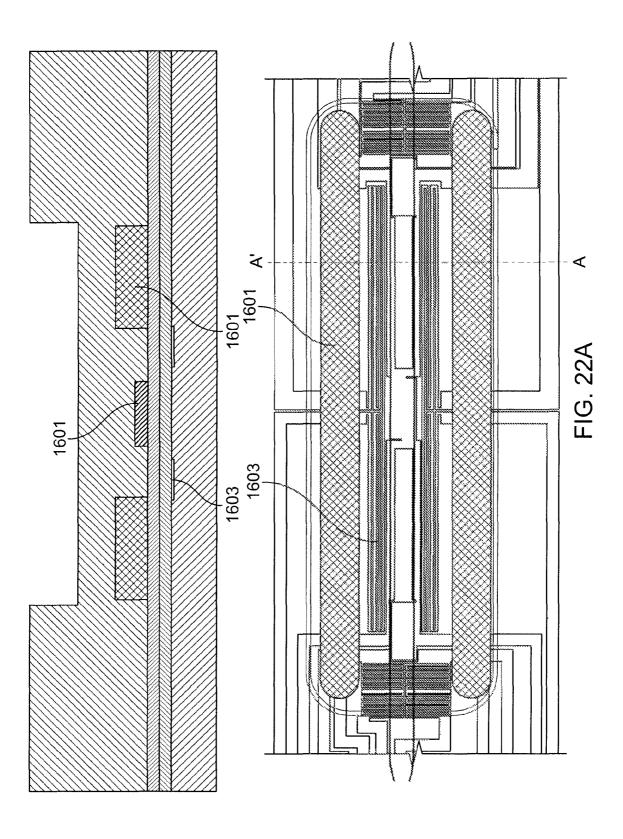


FIG. 20

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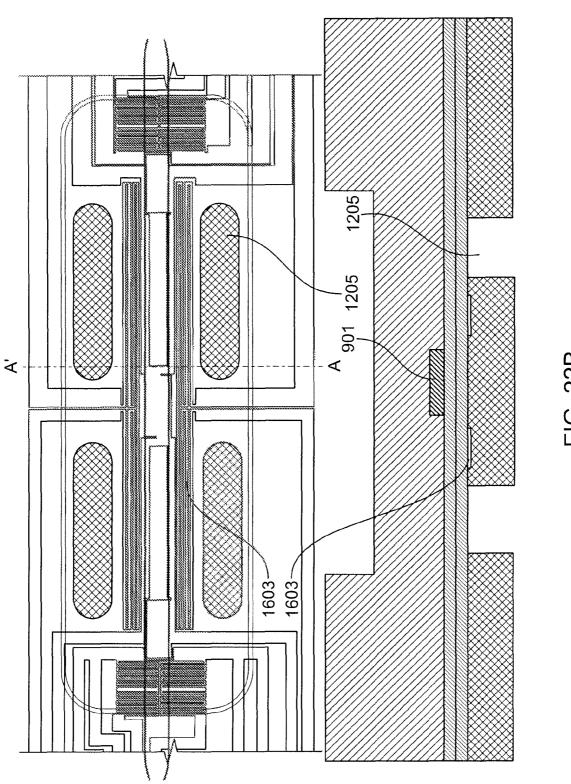


FIG. 22B

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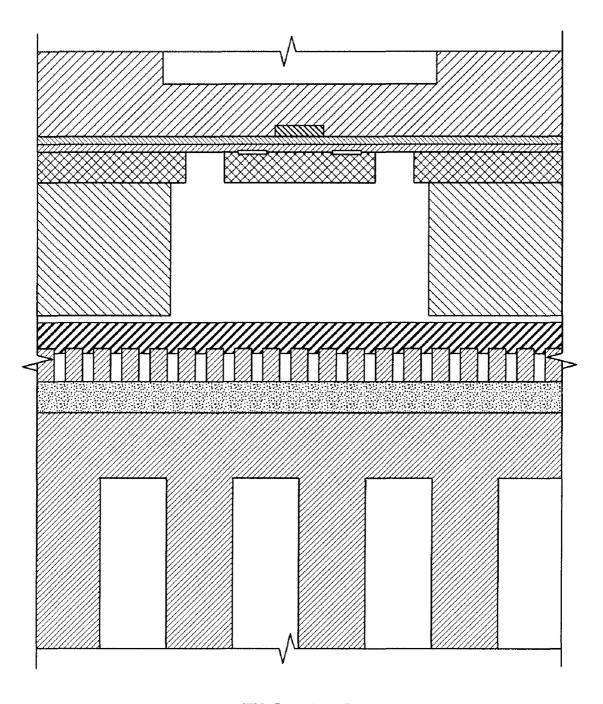
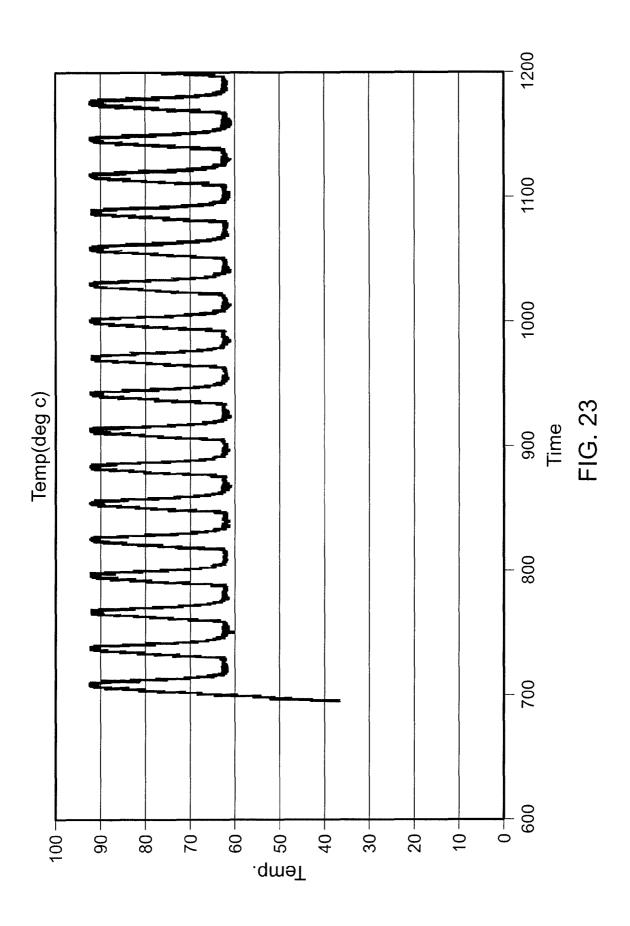


FIG. 22C

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U.S. Patent US 7,998,708 B2 Aug. 16, 2011 **Sheet 34 of 61** 1800 -1802-Laminate Microfluidic Substrate and Trim Edges 1804~ Dispense and Load Wax into the Microvalves 1806~ Inspect Wax is Loaded Properly and Laminate is Adhered Properly to Microfluidic Substrate 1808-Apply Hydrophobic Vent Membrane and Heat Stake to the Top of the Microsubstrate, Trim Edges of the Membrane 1810~ Inspect Membrane is Bonded Well to the Microsubstrate without Heat-clogging the Microchannels 1812~ Apply Thermal Conductive Pad Layer on the Bottom Laminate of the Cartridge 1814~ Apply Two Label Strips on Top of the Microsubstrate, One to Cover the Valves and Second to Protect the Vent Membrane 1816-Print or Apply Small Labels to Show Barcode #, Lot # and **Expiry Date** 1818-

FIG. 24

Stack and Pack Cartridges in Groups of 25

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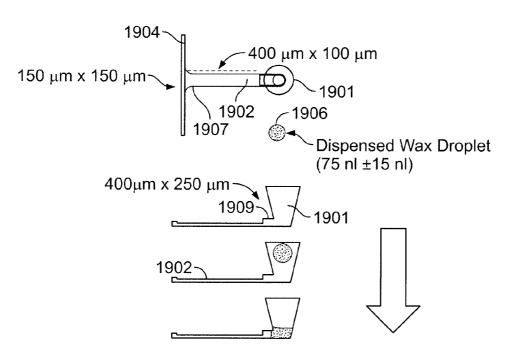
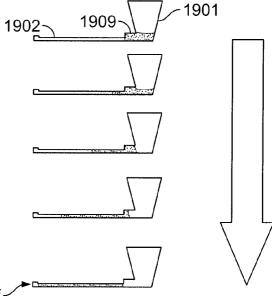


FIG. 25A

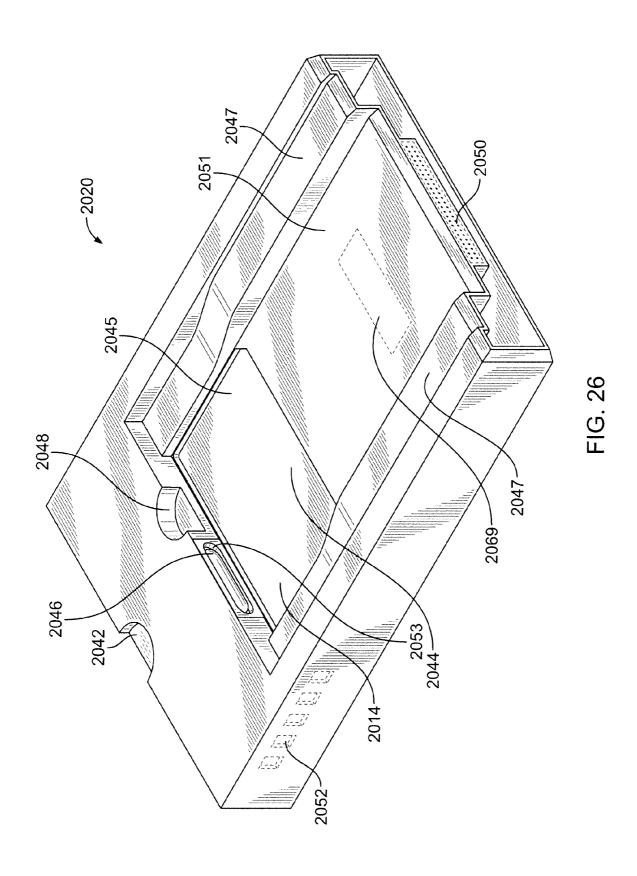


Capillary action of controlled volume of wax causes it to fill up the Wax up to the right interface without blocking the liquid flowable microchannel

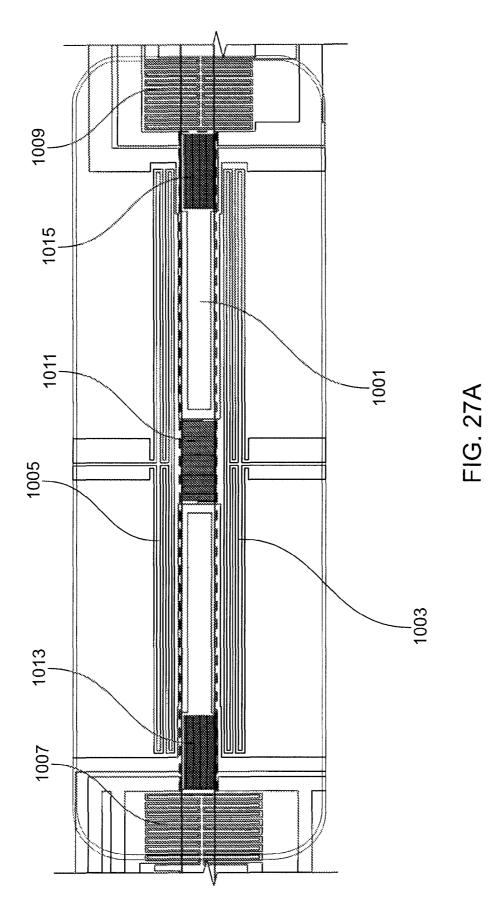
FIG. 25B

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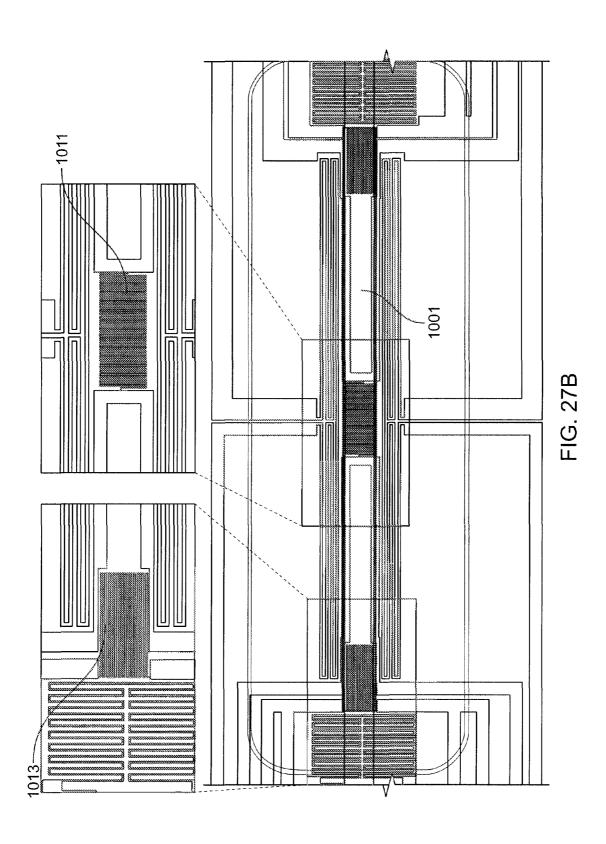


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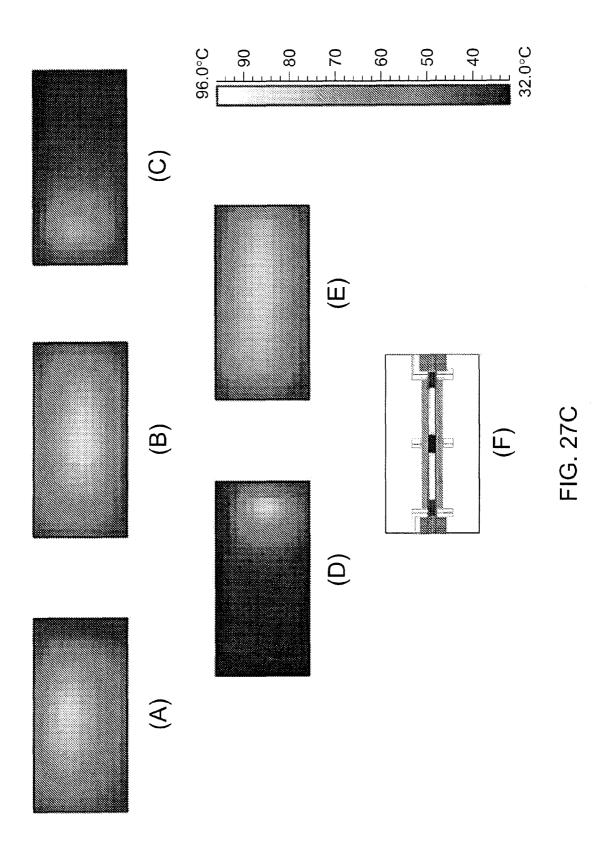
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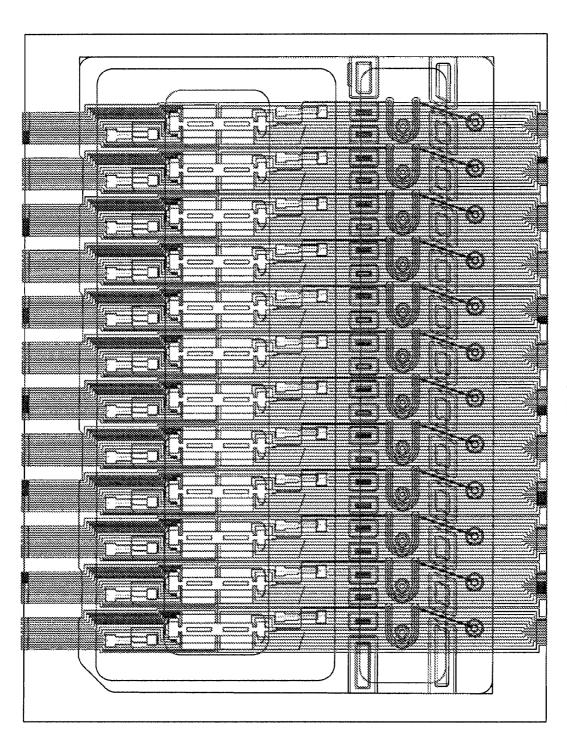


FIG. 28

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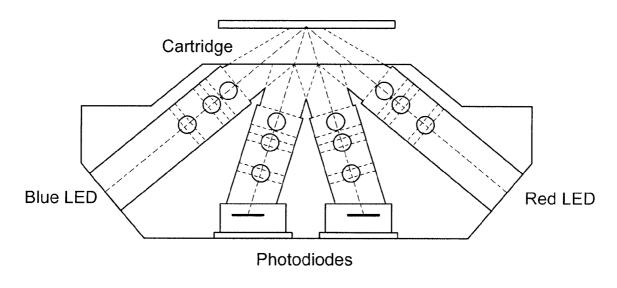
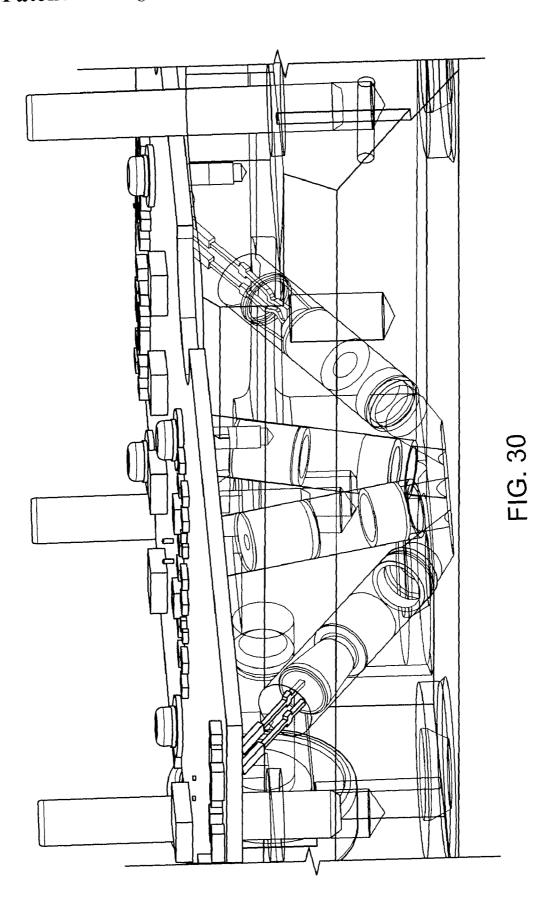
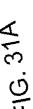
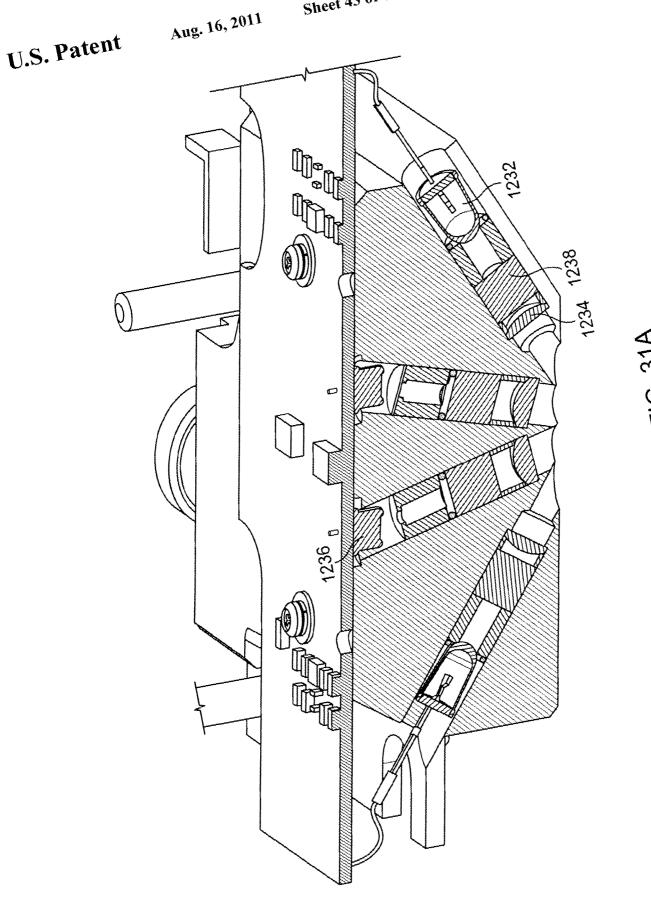


FIG. 29

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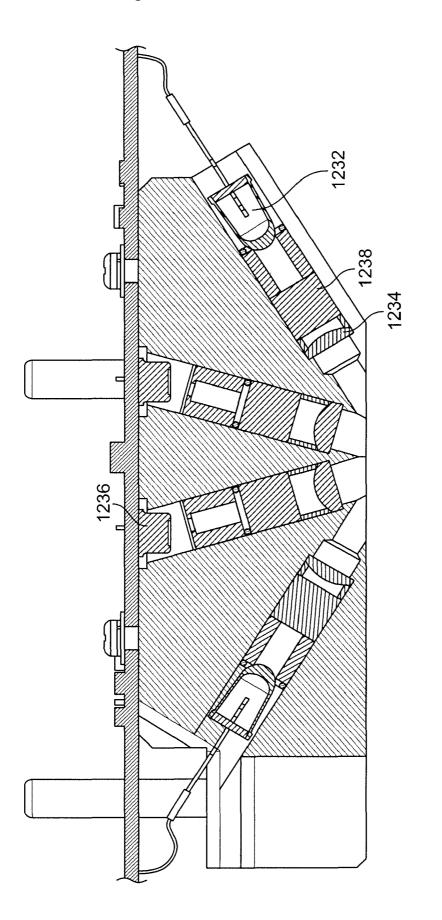


FIG. 31B

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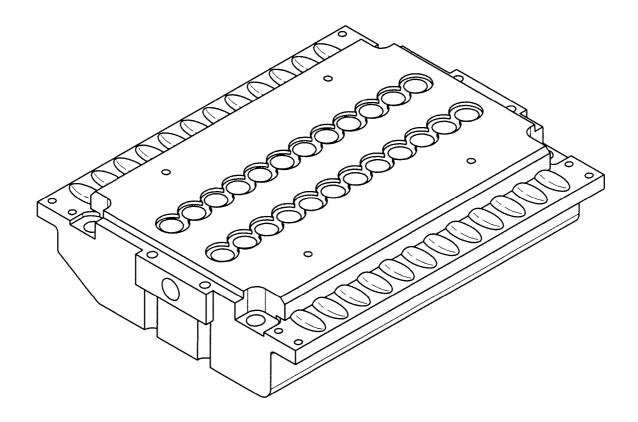
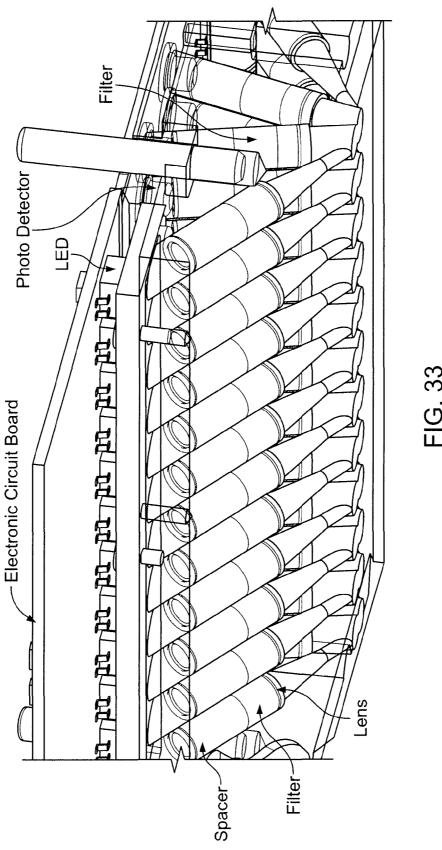


FIG. 32

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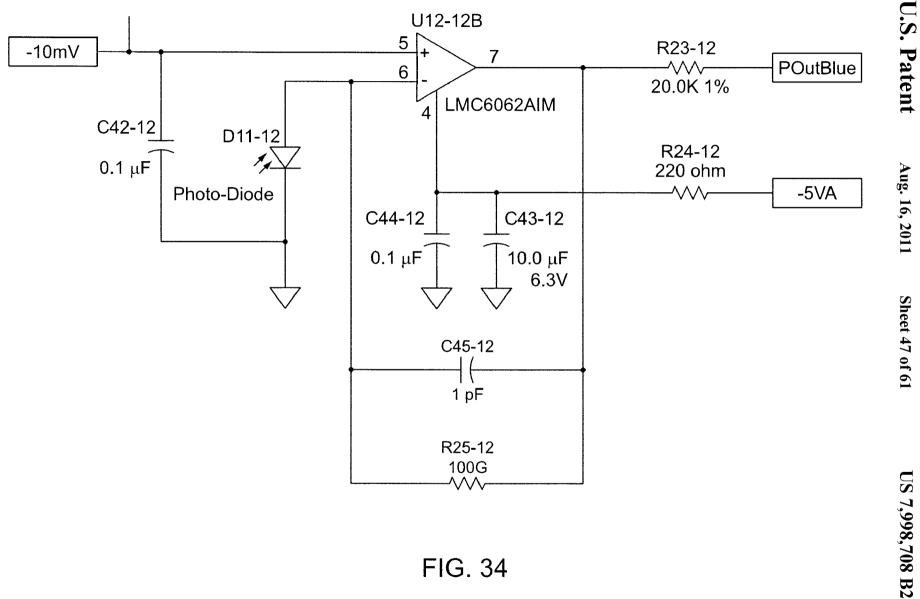
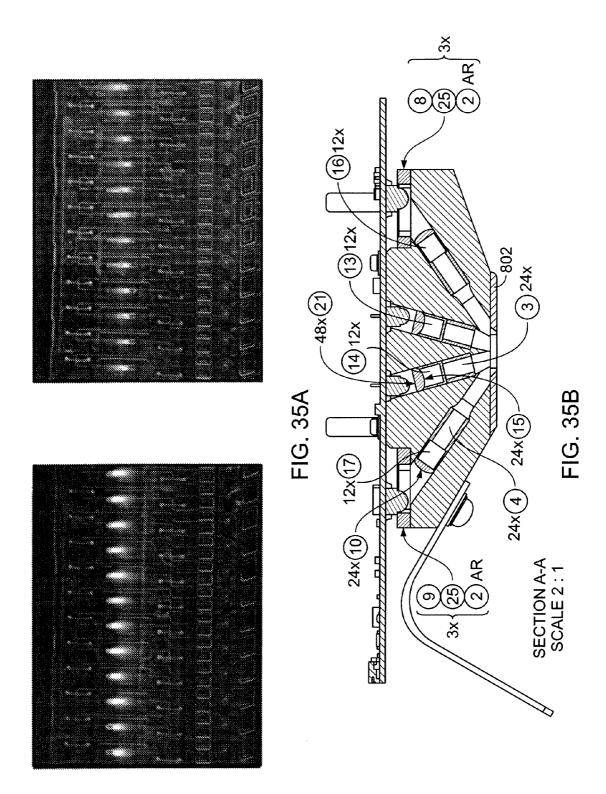


FIG. 34

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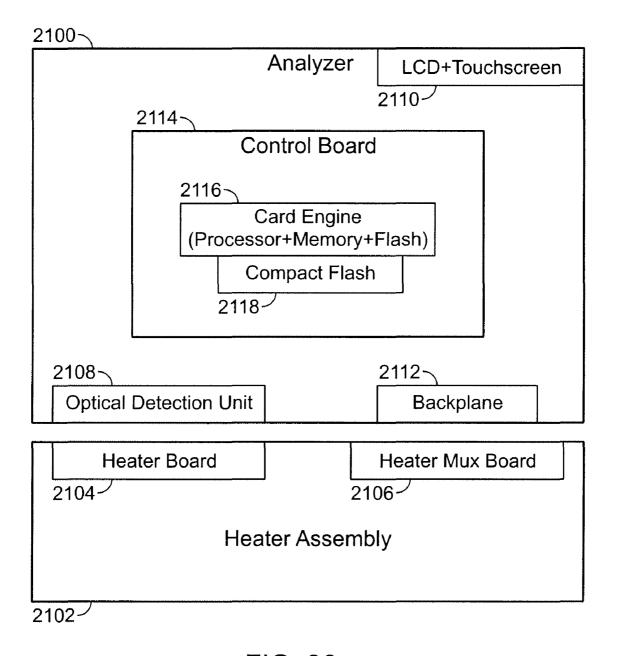


FIG. 36

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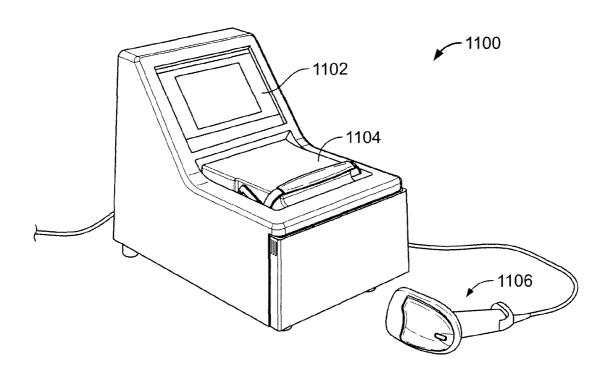


FIG. 37

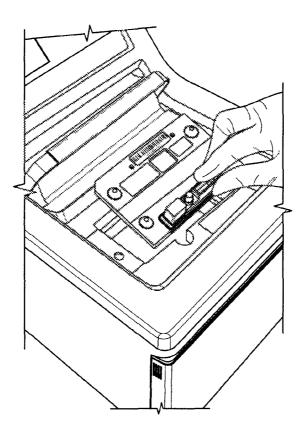
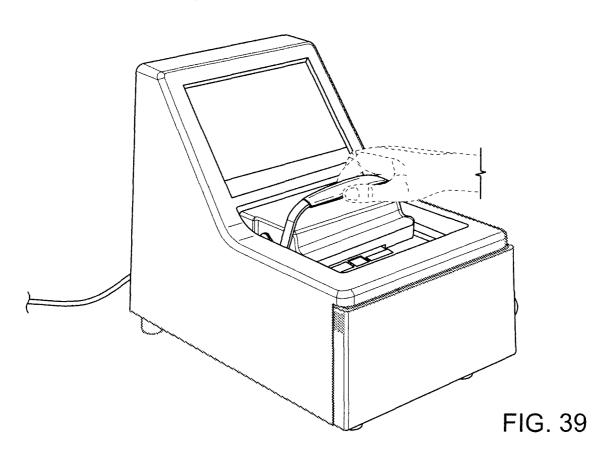


FIG. 38

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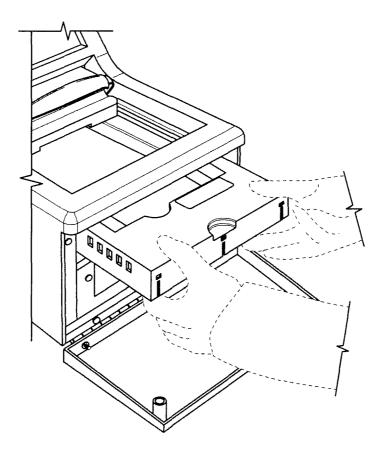


FIG. 40

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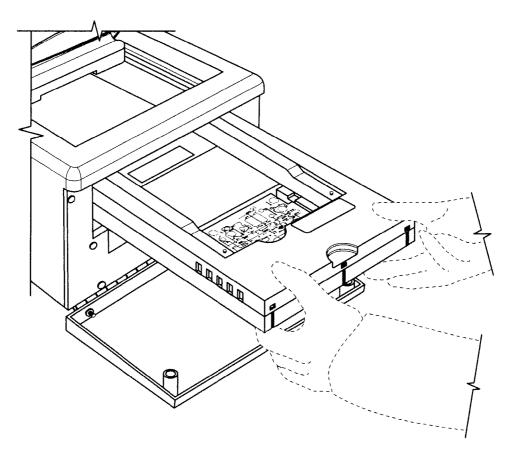


FIG. 41

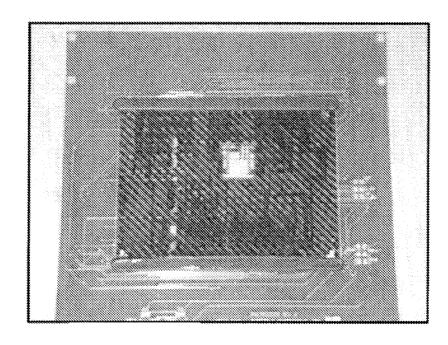


FIG. 42B

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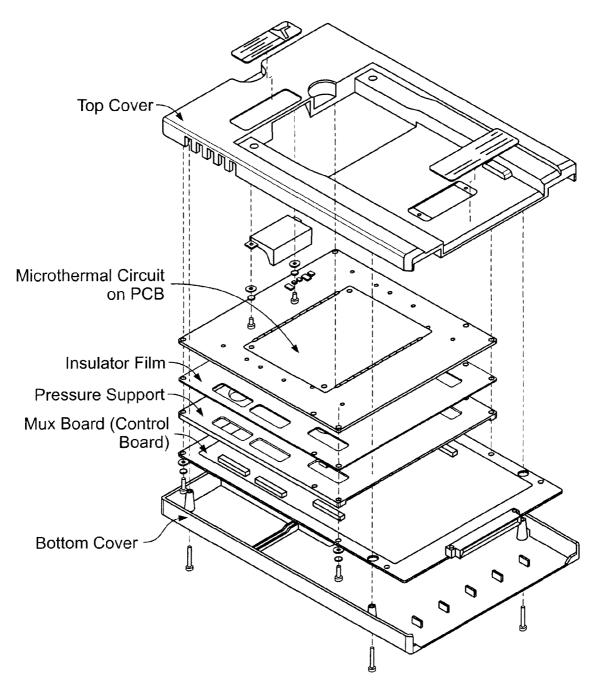
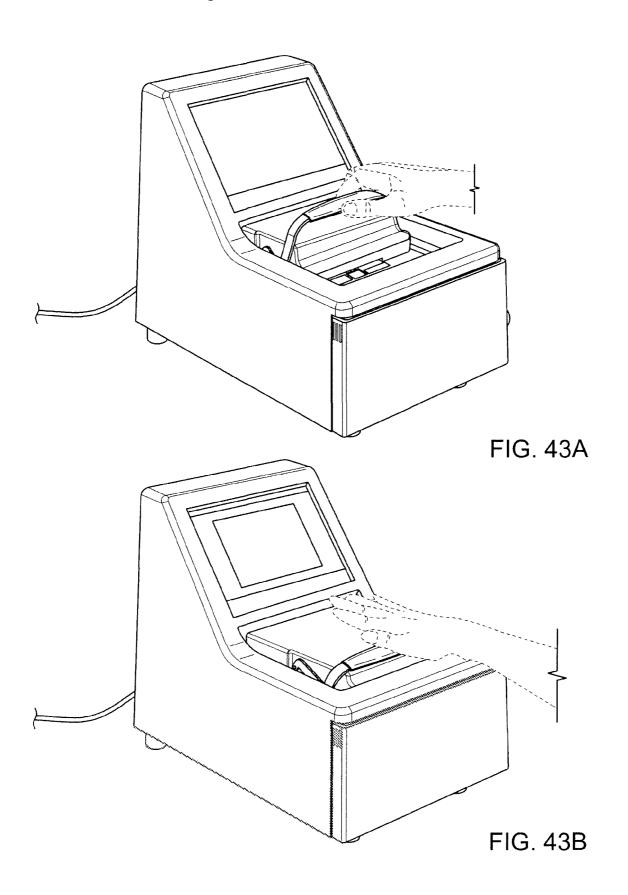


FIG. 42A

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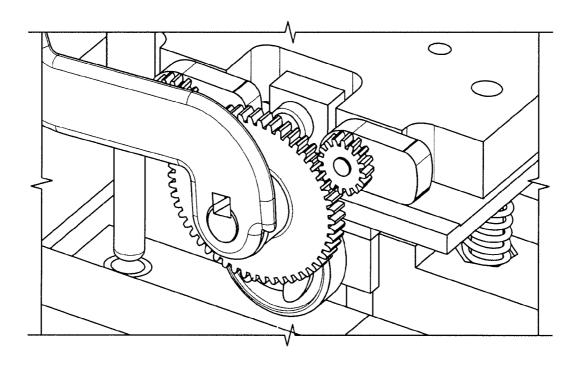


FIG. 44A

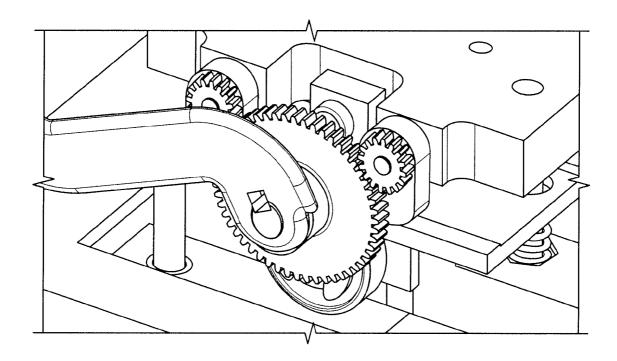


FIG. 44B

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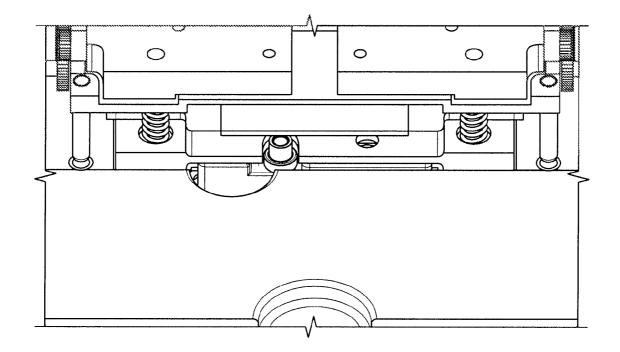
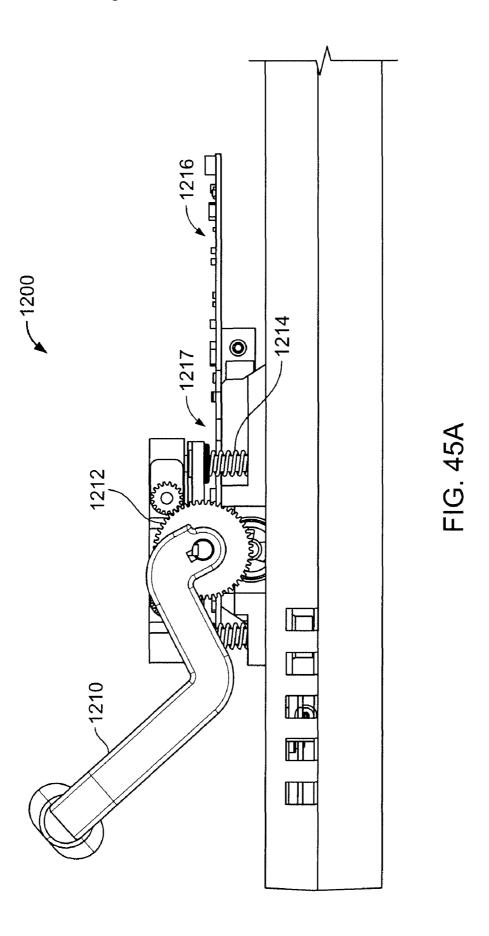


FIG. 44C

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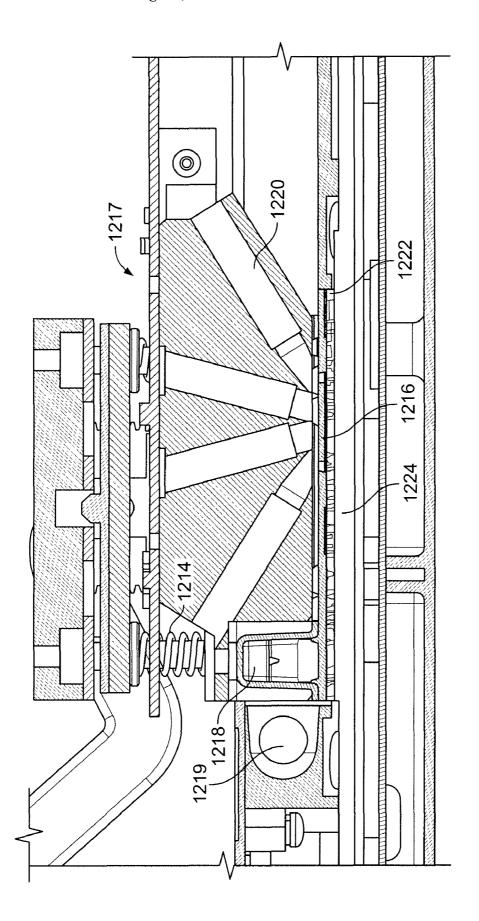


FIG. 450

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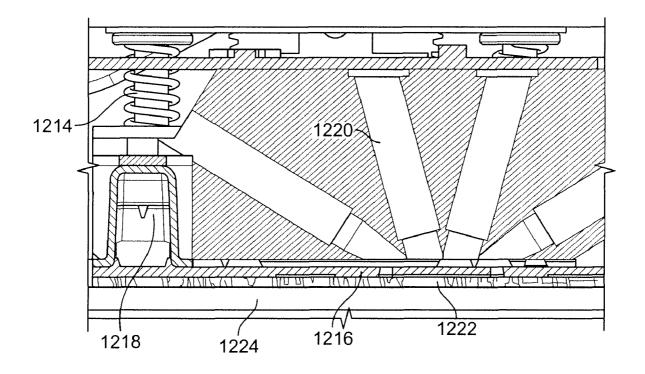


FIG. 45D

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FIG. 46

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### MICROFLUIDIC SYSTEM FOR AMPLIFYING AND DETECTING POLYNUCLEOTIDES IN PARALLEL

#### **CLAIM OF PRIORITY**

The instant application claims the benefit of priority to U.S. provisional applications having Ser. Nos. 60/859,284, filed Nov. 14, 2006, and 60/959,437, filed Jul. 13, 2007, the specifications of both of which are incorporated herein by reference in their entireties. The instant application is also a continuation-in-part of U.S. patent application Ser. No. 11/728, 964, filed Mar. 26, 2007, which claims the benefit of U.S. provisional application Ser. No. 60/786,007, filed Mar. 24, 2006, and U.S. provisional application Ser. No. 60/859,284, filed Nov. 14, 2006. The specification of U.S. patent application Ser. No. 11/728,964 is incorporated herein by reference in its entirety.

#### TECHNICAL FIELD

The technology described herein generally relates to systems for detecting polynucleotides in samples, particularly from biological samples. The technology more particularly 25 relates to microfluidic systems that carry out PCR on nucleotides of interest within microfluidic channels, and detect those nucleotides.

#### BACKGROUND

The medical diagnostics industry is a critical element of today's healthcare infrastructure. At present, however, diagnostic analyses no matter how routine have become a bottleneck in patient care. There are several reasons for this. First, 35 many diagnostic analyses can only be done with highly specialist equipment that is both expensive and only operable by trained clinicians. Such equipment is found in only a few locations—often just one in any given urban area. This means that most hospitals are required to send out samples for analyses to these locations, thereby incurring shipping costs and transportation delays, and possibly even sample loss. Second, the equipment in question is typically not available 'on-demand' but instead runs in batches, thereby delaying the processing time for many samples because they must wait for a 45 machine to fill up before they can be run.

Understanding that sample flow breaks down into several key steps, it would be desirable to consider ways to automate as many of these as possible. For example, a biological sample, once extracted from a patient, must be put in a form suitable for a processing regime that typically involves using PCR to amplify a vector of interest. Once amplified, the presence of a nucleotide of interest from the sample needs to be determined unambiguously. Sample preparation is a process that is susceptible to automation but is also relatively routinely carried out in almost any location. By contrast, steps such as PCR and nucleotide detection have customarily only been within the compass of specially trained individuals having access to specialist equipment.

There is a need for a method and apparatus of carrying out 60 PCR and detection on prepared biological samples, and preferably with high throughput. In particular there is a need for an easy-to-use device that can deliver a diagnostic result on several samples in a short time.

The discussion of the background to the technology herein 65 is included to explain the context of the technology. This is not to be taken as an admission that any of the material

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referred to was published, known, or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word "comprise" and variations thereof, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

#### **SUMMARY**

The present technology addresses systems for detecting polynucleotides in samples, particularly from biological samples. In particular, the technology relates to microfluidic systems that carry out PCR on nucleotides of interest within microfluidic channels, and detect those nucleotides.

An apparatus, comprising: a receiving bay configured to receive a microfluidic cartridge; at least one heat source thermally coupled to the cartridge and configured to carry out PCR on a microdroplet of polynucleotide-containing sample, in the cartridge; a detector configured to detect presence of one or more polynucleotides in the sample; and a processor coupled to the detector and the heat source, configured to control heating of one or more regions of the microfluidic cartridge.

A method of carrying out PCR on a plurality of polynucleotide-containing samples, the method comprising: introducing the plurality of samples in to a microfluidic cartridge, wherein the cartridge has a plurality of PCR reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another; moving the plurality of samples into the respective plurality of PCR reaction chambers; and amplifying polynucleotides contained with the plurality of samples, by application of successive heating and cooling cycles to the PCR reaction chambers.

The details of one or more embodiments of the technology are set forth in the accompanying drawings and further description herein. Other features, objects, and advantages of the technology will be apparent from the description and drawings, and from the claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an exemplary apparatus, a microfluidic cartridge, and a read head, as further described herein;

FIG. 2 shows an exemplary sample-preparation kit;

FIG. 3 shows a schematic diagram of an apparatus;

FIG. 4 shows a cross-section of a pipetting head and a cartridge in position in a microfluidic apparatus.

FIG. 5 shows introduction of a PCR-ready sample into a cartridge, situated in an instrument;

FIGS. 6A-6E show exemplary embodiments of an apparatus:

FIG. 7 shows an exploded view of an apparatus;

FIG. 8 shows a block diagram of control circuitry;

FIG. 9 shows a plan view of an exemplary multi-lane microfluidic cartridge;

FIG. 10A shows an exemplary multi-lane cartridge;

FIG. 10B shows a portion of an exemplary multi-lane cartridge;

FIGS. 11A-C show exploded view of an exemplary microfluidic cartridge;

FIG. 12 shows an exemplary highly-multiplexed microfluidic cartridge;

FIGS. 13-16 show various aspects of exemplary highly multiplexed microfluidic cartridges; and

FIGS. 17A-C show various aspects of a radially configured highly multiplexed microfluidic cartridge.

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FIG. 18 shows an exemplary microfluidic network in a lane of a multi-lane cartridge;

FIGS. 19A-19D show exemplary microfluidic valves;

FIG. 20 shows an exemplary bubble vent;

FIG. 21 shows a cross-section of a microfluidic cartridge, 5 when in contact with a heater substrate;

FIGS. **22**A-**22**C shows various cut-away sections that can be used to improve cooling rates during PCR thermal cycling;

FIG. 23 shows a plot of temperature against time during a PCR process, as performed on a microfluidic cartridge as <sup>10</sup> described herein;

FIG. 24 shows an assembly process for a cartridge as further described herein:

FIGS. **25**A and **25**B show exemplary deposition of wax droplets into microfluidic valves;

FIG. 26 shows an exemplary heater unit;

FIGS. 27A and 27B show a plan view of heater circuitry adjacent to a PCR reaction chamber;

FIG. 27C shows thermal images of heater circuitry in operation:

FIG. 28 shows an overlay of an array of heater elements on an exemplary multi-lane microfluidic cartridge, wherein various microfluidic networks are visible;

FIG. **29** shows a cross-sectional view of an exemplary detector;

FIG. 30 shows a perspective view of a detector in a readhead:

FIG. 31A, 31B shows a cutaway view of an exemplary detector in a read-head:

FIG. **32** shows an exterior view of an exemplary multiplexed read-head with an array of detectors therein;

FIG. 33 shows a cutaway view of an exemplary multiplexed read-head, as in FIG. 18;

FIG. 34 shows exemplary pre-amplifier circuitry for a fluorescence detector;

FIG. 35A shows effects of aperturing on fluorescence intensity; FIG. 35B shows a detector in cross section with an exemplary aperture;

FIG. 36 shows an exemplary layout for electronics and software components, as further described herein;

FIG. 37 shows an exemplary apparatus, a microfluidic cartridge, and a read head, as further described herein;

FIGS. **38-39** show positioning of a cartridge in an exemplary apparatus;

FIGS. **40** and **41** show removal of a heater unit from an <sup>45</sup> exemplary apparatus;

FIGS. 42A and 42B show an exemplary heater unit and heater substrate;

FIGS. **43**A and **43**B show an exemplary apparatus having a detector mounted in a sliding lid;

FIGS. 44A-44C show a force member;

FIGS. 45A-45D show a force member associated with a detector;

FIG. 46 shows a block diagram of exemplary electronic circuitry in conjunction with a detector as described herein; 55 analyses described herein is shown in FIG. 3. The geometric

Additional figures are illustrated within the examples, and are further described therein.

Like reference symbols in the various drawings indicate like elements.

### DETAILED DESCRIPTION

Overview of Apparatus

The present technology relates to a system and related methods for amplifying, and carrying out diagnostic analyses 65 on, polynucleotides (e.g., a DNA, RNA, mRNA, or rRNA) from biological samples. For example, the system and meth-

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ods can determine whether a polynucleotide indicative of the presence of a particular pathogen (such as a bacterium or a virus) can be present. The polynucleotide may be a sample of genomic DNA, or may be a sample of mitochondrial DNA. The nucleotides are typically provided to the system having been isolated or released from particles such as cells in the sample. The system includes a disposable microfluidic cartridge containing multiple sample lanes in parallel and a reusable instrument platform (a PCR analyzer apparatus) that can actuate on-cartridge operations, can detect (e.g., by fluorescence detection) and analyze the products of the PCR amplification in each of the lanes separately, in all simultaneously, or in groups simultaneously, and, optionally, can display the results on a graphical user interface.

A system, microfluidic cartridge, heater unit, detector, kit, methods, and associated computer program product, are now further described.

By cartridge is meant a unit that may be disposable, or reusable in whole or in part, and that is configured to be used in conjunction with some other apparatus that has been suitably and complementarily configured to receive and operate on (such as deliver energy to) the cartridge.

By microfluidic, as used herein, is meant that volumes of sample, and/or reagent, and/or amplified polynucleotide are from about 0.1 µl to about 999 µl, such as from 1-100 µl, or from 2-25 µl. Similarly, as applied to a cartridge, the term microfluidic means that various components and channels of the cartridge, as further described herein, are configured to accept, and/or retain, and/or facilitate passage of microfluidic volumes of sample, reagent, or amplified polynucleotide.

FIG. 1 shows a perspective view of an exemplary apparatus 100 consistent with those described herein, as well as various components thereof, such as exemplary cartridge 200 that contains multiple sample lanes, and exemplary read head 300 that contains detection apparatus for reading signals from cartridge 200. The apparatus 100 of FIG. 1 is able to carry out real-time PCR on a number of samples in cartridge 200 simul-40 taneously. Preferably the number of samples is 12 samples, as illustrated with exemplary cartridge 200, though other numbers of samples such as 4, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 are within the scope of the present description. In preferred operation of the apparatus, a PCR-ready solution containing the sample, and, optionally, one or more analytespecific reagents (ASR's) is prepared, as further described elsewhere (see, e.g., U.S. patent application publication 2006-0166233, incorporated herein by reference), prior to introduction into cartridge 200. An exemplary kit for preparing a PCR-ready sample, for use with the system described herein, the kit comprising buffers, lysis pellets, and affinity pellets, is shown in FIG. 2.

A schematic overview of a system 981 for carrying out analyses described herein is shown in FIG. 3. The geometric arrangement of the components of system 981 shown in FIG. 3, as well as their respective connectivities, is exemplary and not intended to be limiting.

System Overview

A processor 980, such as a microprocessor, is configured to control functions of various components of the system as shown, and is thereby in communication with each such component. In particular, processor 980 is configured to receive data about a sample to be analyzed, e.g., from a sample reader 990, which may be a barcode reader, an optical character reader, or an RFID scanner (radio frequency tag reader). For example, the sample identifier can be a handheld bar code reader. Processor 980 can be configured to accept user

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instructions from an input **984**, where such instructions may include instructions to start analyzing the sample, and choices of operating conditions.

Processor 980 can also be configured to communicate with an optional display 982, so that, for example, information 5 including but not limited to the current status of the system, progress of PCR thermocycling, and any warning message in case of malfunction of either system or cartridge, as well as results of analysis, are transmitted to the display. Additionally, processor 980 may transmit one or more questions to be 10 displayed on display 982 that prompt a user to provide input in response thereto. Thus, in certain embodiments, input 984 and display 982 are integrated with one another.

Processor 980 can be optionally further configured to transmit results of an analysis to an output device such as a 15 printer, a visual display, or a speaker, or a combination thereof, the transmission being either directly through a directly dedicated printer cable, or wirelessly, or via a network connection.

Processor 980 is still further optionally connected via a 20 communication interface such as a network interface to a computer network 988. The communication interface can be one or more interfaces selected from the group consisting of: a serial connection, a parallel connection, a wireless network connection and a wired network connection such as an eth- 25 ernet, firewire, cable connection, or one using USB connectivity. Thereby, when the system is suitably addressed on the network, a remote user may access the processor and transmit instructions, input data, or retrieve data, such as may be stored in a memory (not shown) associated with the processor, or on 30 some other computer-readable medium that is in communication with the processor. The computer network connection may also permit extraction of data to a remote location, such as a personal computer, personal digital assistant, or network storage device such as computer server or disk farm. The 35 apparatus may further be configured to permit a user to e-mail results of an analysis directly to some other party, such as a healthcare provider, or a diagnostic facility, or a patient.

Although not shown in FIG. 3, in various embodiments, input 984 can include one or more input devices selected from 40 the group consisting of: a keyboard, a touch-sensitive surface, a microphone, a track-pad, and a mouse. A suitable input device may further comprise a reader of formatted electronic media, such as, but not limited to, a flash memory card, memory stick, USB-stick, CD, or floppy diskette. An input 45 device may further comprise a security feature such as a fingerprint reader, retinal scanner, magnetic strip reader, or bar-code reader, for ensuring that a user of the system is in fact authorized to do so, according to, for example, pre-loaded identifying characteristics of authorized users. An input 50 device may additionally—and simultaneously—function as an output device for writing data in connection with sample analysis. For example, if an input device is a reader of formatted electronic media, it may also be a writer of such media. Data that may be written to such media by such a 55 device includes, but is not limited to, environmental information, such as temperature or humidity, pertaining to an analysis, as well as a diagnostic result, and identifying data for the sample in question.

Additionally, in various embodiments, the apparatus can 60 further comprise a data storage medium configured to receive data from one or more of the processor, an input device, and a communication interface, the data storage medium being one or more media selected from the group consisting of: a hard disk drive, an optical disk drive, or one or more removable storage media such as a CD-R, CD-RW, USB-drive, and a flash card.

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Processor 980 is further configured to control various aspects of sample diagnosis, as follows in overview, and as further described in detail herein. The system is configured to operate in conjunction with a complementary cartridge 994, such as a microfluidic cartridge. The cartridge is itself configured, as further described herein, to receive one or more samples 996 containing one or more polynucleotides in a form suitable for amplification and diagnostic analysis. The cartridge has dedicated regions within which amplification, such as by PCR, of the polynucleotides is carried out when the cartridge is situated in the apparatus.

The microfluidic cartridge is received by a receiving bay 992 configured to selectively receive the cartridge. For example, the receiving bay and the microfluidic cartridge can be complementary in shape so that the microfluidic cartridge is selectively received in, e.g., a single orientation. The microfluidic cartridge can have a registration member that fits into a complementary feature of the receiving bay. The registration member can be, for example, a cut-out on an edge of the cartridge, such as a corner that is cut-off, or one or more notches that are made on one or more of the sides. By selectively receiving the cartridge, the receiving bay can help a user to place the cartridge so that the apparatus can properly operate on the cartridge. The receiving bay can also be configured so that various components of the apparatus that can operate on the microfluidic cartridge (heat sources, detectors, force members, and the like) are positioned to properly operate on the microfluidic cartridge. In some embodiments, the apparatus can further include a sensor coupled to the processor, the sensor configured to sense whether the microfluidic cartridge is selectively received.

The receiving bay is in communication with a heater unit 998 that itself is controlled by processor 980 in such a way that specific regions of the cartridge, such as individual sample lanes, are independently and selectively heated at specific times during amplification and analysis. The processor can be configured to control application of heat to the individual sample lanes, separately, in all simultaneously, or in groups simultaneously.

The heat source can be, for example, a contact heat source such as a resistive heater or a network of resistive heaters, or a Peltier device, and the like. The contact heat source can be configured to be in direct physical contact with one or more distinct locations of a microfluidic cartridge received in the receiving bay. In various embodiments, each contact source heater can be configured to heat a distinct location having an average diameter in 2 dimensions from about 1 millimeter (mm) to about 15 mm (typically about 1 mm to about 10 mm), or a distinct location having a surface area of between about 1 mm² about 225 mm² (typically between about 1 mm² and about 100 mm², or in some embodiments between about 5 mm² and about 50 mm²).

In various embodiments, the heat source can be situated in an assembly that is removable from the apparatus, for example, to permit cleaning or to replace the heater configuration.

In various embodiments, the apparatus can include a compliant layer at the contact heat source configured to thermally couple the contact heat source with at least a portion of a microfluidic cartridge received in the receiving bay. The compliant layer at can have a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100.

In various embodiments, the apparatus can further include one or more force members (not shown in FIG. 3) configured

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to apply force to thermally couple the at least one heat source at least a portion of a microfluidic cartridge received in the receiving bay.

In various embodiments, the one or more force members are configured to apply force to a plurality of locations in the microfluidic cartridge. The force applied by the one or more force members can result in an average pressure at an interface between a portion of the receiving bay and a portion of the microfluidic cartridge of between about 5 kilopascals and about 50 kilopascals, for example, the average pressure can be at least about 7 kilopascals, and still more preferably at least about 14 kilopascals. At least one force member can be manually operated. At least one force member can be mechanically coupled to a lid at the receiving bay, whereby operation of the lid operates the force member. The application of force is important to ensure consistent thermal contact between the heater wafer and the PCR reactor and microvalves in the microfluidic cartridge.

In various embodiments, the apparatus can further include 20 a lid at the receiving bay, the lid being operable to at least partially exclude ambient light from the receiving bay. The lid can be, for example, a sliding lid. The lid can include the optical detector. A major face of the lid at the optical detector or at the receiving bay can vary from planarity by less than 25 about 100 micrometers, for example, less than about 25 micrometers. The lid can be configured to be removable from the apparatus. The lid can include a latching member that ensures that the lid is securely closed before amplification reactions are applied to the samples in the cartridge.

The processor is also configured to receive signals from and control a detector **999** configured to detect a polynucleotide in a sample in one or more of the individual sample lanes, separately or simultaneously. The processor thereby provides an indication of a diagnosis from the cartridge **994**. Diagnosis can be predicated on the presence or absence of a specific polynucleotide in a particular sample. The diagnosis can be transmitted to the output device **986** and/or the display **982**, as described hereinabove.

The detector can be, for example, an optical detector that includes a light source that selectively emits light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent 45 polynucleotide probe or a fragment thereof. Alternatively, for example, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent 50 dye; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof; or for example, the optical detector can be 55 configured to independently detect a plurality of fluorescent dyes at a plurality of different locations on a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof in a different sample.

A suitable processor **980** can be designed and manufactured according to, respectively, design principles and semi-conductor processing methods known in the art.

The system in FIG. 3 is configured so that a cartridge with capacity to receive multiple samples can be acted upon by the 65 system to analyze multiple samples—or subsets thereof—simultaneously, or to analyze the samples consecutively. It is

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also consistent that additional samples can be added to a cartridge, while previously added samples are being amplified and analyzed.

The system shown in outline in FIG. 3, as with other exemplary embodiments described herein, is advantageous at least because it does not require locations within the system suitably configured for storage of reagents. Neither does the system, or other exemplary embodiments herein, require inlet or outlet ports that are configured to receive reagents from, e.g., externally stored containers such as bottles, canisters, or reservoirs. Therefore, the system in FIG. 3 is self-contained and operates in conjunction with a microfluidic cartridge, wherein the cartridge has locations within it configured to receive mixtures of sample and PCR reagents.

The system of FIG. 3 may be configured to carry out operation in a single location, such as a laboratory setting, or may be portable so that it can accompany, e.g., a physician, or other healthcare professional, who may visit patients at different locations. The system is typically provided with a power-cord so that it can accept AC power from a mains supply or generator. An optional transformer (not shown) built into the system, or situated externally between a power socket and the system, transforms AC input power into a DC output for use by the system. The system may also be configured to operate by using one or more batteries and therefore is also typically equipped with a battery recharging system, and various warning devices that alert a user if battery power is becoming too low to reliably initiate or complete a diagnostic analysis.

The system of FIG. 3 may further be configured, for multiplexed cartridge analysis. In one such configuration, multiple instances of a system, as outlined in FIG. 3, are operated in conjunction with one another to accept and to process multiple cartridges, where each cartridge has been loaded with a different sample. Each component shown in FIG. 3 may therefore be present as many times as there are cartridges, though the various components may be configured in a common housing.

In still another configuration, a system is configured to accept and to process multiple cartridges, but one or more components in FIG. 3 is common to multiple cartridges. For example, a single device may be configured with multiple cartridge receiving bays, but a common processor and user interface suitably configured to permit concurrent, consecutive, or simultaneous, control of the various cartridges. In such an embodiment a single detector, for example, can scan across all of the multiple cartridges. It is further possible that such an embodiment, also utilizes a single sample reader, and a single output device.

In still another configuration, a system as shown in FIG. 3 is configured to accept a single cartridge, but wherein the single cartridge is configured to process more than 1, for example, 2, 3, 4, 5, or 6, samples in parallel, and independently of one another.

It is further consistent with the present technology that a cartridge can be tagged, e.g., with a molecular bar-code indicative of one or more of the samples, to facilitate sample tracking, and to minimize risk of sample mix-up. Methods for such tagging are described elsewhere, e.g., in U.S. patent application publication Ser. No. 10/360,854, incorporated herein by reference.

In various embodiments, the apparatus can further include an analysis port. The analysis port can be configured to allow an external sample analyzer to analyze a sample in the microfluidic cartridge; for example, the analysis port can be a hole or window in the apparatus which can accept an optical detection probe that can analyze a sample in situ in the microfluidic

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cartridge. In some embodiments, the analysis port can be configured to direct a sample from the microfluidic cartridge to an external sample analyzer; for example, the analysis port can include a conduit in fluid communication with the microfluidic cartridge that direct a liquid sample to a chromatography apparatus, an optical spectrometer, a mass spectrometer, or the like.

Apparatus 100 may optionally comprise one or more stabilizing feet that cause the body of the device to be elevated above a surface on which system 100 is disposed, thereby permitting ventilation underneath system 100, and also providing a user with an improved ability to lift system 100. There may be 2, 3, 4, 5, or 6, or more feet, depending upon the size of system 100. Such feet are preferably made of rubber, or plastic, or metal, and in some embodiments may elevate the 15 body of system 100 by from about 2 to about 10 mm above a surface on which it is situated. The stabilizing function can also be provided by one or more runners that run along one or more edges-or are inwardly displaced from one or more edges-of the underside of the apparatus. Such runners can 20 also be used in conjunction with one or more feet. In another embodiment, a turntable situated on the underside permits the apparatus to be rotated in a horizontal or near-horizontal plane when positioned on, e.g., a benchtop, to facilitate access from a number of angles by a user.

FIG. 4 shows a schematic cross-sectional view of a part of an apparatus as described herein, showing input of sample into a cartridge 200 via a pipette 10 (such as a disposable pipette) and an inlet 202. Inlet 202 is preferably configured to receive a pipette or the bottom end of a PCR tube and thereby accept sample for analysis with minimum waste, and with minimum introduction of air. Cartridge 200 is disposed on top of and in contact with a heater substrate 400. Read head 300 is positioned above cartridge 200 and a cover for optics 310 restricts the amount of ambient light that can be detected by 35 the read head.

FIG. 5 shows an example of 4-pipette head used for attaching disposable pipette tips, prior to dispensing PCR-ready sample into a cartridge.

**Exemplary Systems** 

FIGS. 6A-6E show exterior perspective views of various configurations of an exemplary system, as further described herein. FIG. 6A shows a perspective view of a system 2000 for receiving microfluidic cartridge (not shown), and for causing and controlling various processing operations to be performed a sample introduced into the cartridge. The elements of system 2000 are not limited to those explicitly shown. For example, although not shown, system 2000 may be connected to a hand-held bar-code reader, as further described herein.

System 2000 comprises a housing 2002, which can be 50 made of metal, or a hardened plastic. The form of the housing shown in FIG. 6A embodies stylistic as well as functional features. Other embodiments of the technology may appear somewhat differently, in their arrangement of the components, as well as their overall appearance, in terms of smoothness of lines, and of exterior finish, and texture. System 2000 further comprises one or more stabilizing members 2004. Shown in FIG. 6A is a stabilizing foot, of which several are normally present, located at various regions of the underside of system 2000 so as to provide balance and support. For 60 example, there may be three, four, five, six, or eight such stabilizing feet. The feet may be moulded into and made of the same material as housing 2002, or may be made of one or more separate materials and attached to the underside of system 2000. For example, the feet may comprise a rubber 65 that makes it hard for system 2000 to slip on a surface on which it is situated, and also protects the surface from

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scratches. The stabilizing member of members may take other forms than feet, for example, rails, runners, or one or more pads

System 2000 further comprises a displsy 2006, which may be a liquid crystal display, such as active matrix, an OLED, or some other suitable form. It may present images and other information in color or in black and white. Display 2006 may also be a touch-sensitive display and therefore may be configured to accept input from a user in response to various displayed prompts. Display 2006 may have an anti-reflective coating on it to reduce glare and reflections from overhead lights in an laboratory setting. Display 2006 may also be illuminated from e.g., a back-light, to facilitate easier viewing in adark laboratory.

System 2000, as shown in FIG. 6A, also comprises a moveable lid 2010, having a handle 2008. The lid 2010 can slide back and forward. In FIG. 6A, the lid is in a forward position, whereby it is "closed". In FIG. 6B, the lid is shown in a back position, wherein the lid is "open" and reveals a receiving bay 2014 that is configured to receive a microfluidic cartridge. Of course, as one of ordinary skill in the art would appreciate, the technology described herein is not limited to a lid that slides, or one that slides back and forward. Side to side movement is also possible, as is a configuration where the lid is "open" when positioned forward in the device. It is also possible that the lid is a hinged lid, or one that is totally removable.

Handle 2008 performs a role of permitting a user to move lid 2010 form one position to another, and also performs a role of causing pressure to be forced down on the lid, when in a closed position, so that pressure can be applied to a cartridge in the receiving bay 2014. In FIG. 6C, handle 2008 is shown in a depressed position, wherein force is thereby applied to lid 2014, and thus pressure is applied to a cartridge received in the receiving bay beneath the lid.

In one embodiment, the handle and lid assembly are also fitted with a mechanical sensor that does not permit the handle to be depressed when there is no cartridge in the receiving bay. In another embodiment, the handle and lid assembly are fitted with a mechanical latch that does not permit the handle to be raised when an analysis is in progress.

A further configuration of system 2000 is shown in FIG. 6D, wherein a door 2012 is in an open position. Door 2012 is shown in a closed position in FIGS. 6A-C. The door is an optional component that permits a user to access a heater module 2020, and also a computer-readable medium input tray 2022. System 2000 can function without a door that covers heater module 2020 and medium input 2022, but such a door has convenience attached to it. Although the door 2012 is shown hinged at the bottom, it may also be hinged at one of its sides, or at its upper edge. Door 2012 may alternatively be a removable cover, instead of being hinged. Door 2012, may also be situated at the rear, or side of system 2000 for example, if access to the heater module and/or computer readable medium input is desired on a different face of the system. It is also consistent with the system herein that the heater module, and the computer readable medium input are accessed by separate doors on the same or different sides of the device, and wherein such separate doors may be independently hinged or removable.

Heater module 2020 is preferably removable, and is further described hereinbelow.

Computer readable medium input 2022 may accept one or more of a variety of media. Shown in FIG. 2D is an exemplary form of input 2022, a CD-Rom tray for accepting a CD, DVD, or mini-CD, or mini-DVD, in any of the commonly used readable, read-writable, and writable formats. Also consistent with the description herein is an input that can accept another

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form of medium, such as a floppy disc, flash memory such as memory stick, compact flash, smart data-card, or secure-data card, a pen-drive, portable USB-drive, zip-disk, and others. Such an input can also be configured to accept several different forms of media. Such an input 2022 is in communication with a processor (as described in connection with FIG. 3, though not shown in FIGS. 6A-E), that can read data from a computer-readable medium when properly inserted into the input.

FIG. 6E shows a plan view of a rear of system 2000. Shown 10 are an air vent 2024, or letting surplus heat escape during an analysis. Typically, on the inside of system 2000, and by air vent 2024 and not shown in FIG. 6E, is a fan. Other ports shown in FIG. 6E are as follows: a power socket 2026 for accepting a power cord that will connect system 2000 to a 15 supply of electricity; an ethernet connection 2028 for linking system 2000 to a computer network such as a local area network; an phone-jack connection 2032 for linking system 2000 to a communication network such as a telephone network; one or more USB ports 2030, for connecting system 20 2000 to one or more peripheral devices such as a printer, or a computer hard drive; an infra-red port for communicating with, e.g., a remote controller (not shown), to permit a user to control the system without using a touch-screen interface. For example, a user could remotely issue scheduling commands 25 to system 2000 to cause it to start an analysis at a specific time in the future.

Features shown on the rear of system 2000 may be arranged in any different manner, depending upon an internal configuration of various components. Additionally, features 30 shown as being on the rear of system 2000, may be optionally presented on another face of system 2000, depending on design preference. Shown in FIG. 6E are exemplary connections. It would be understood that various other features, including inputs, outputs, sockets, and connections, may be 35 present on the rear face of system 2000, though not shown, or on other faces of system 2000.

An exploded view of an exemplary embodiment of the apparatus is shown in FIG. 7, particularly showing internal features of apparatus 2000. Apparatus 2000 can comprise a 40 computer readable medium configured with hardware/firmware that can be employed to drive and monitor the operations on a cartridge used therewith, as well as software to interpret, communicate and store the results of a diagnostic test performed on a sample processed in the cartridge. Referring to 45 FIG. 7, typical components of the apparatus 2000 are shown and include, for example, control electronics 2005, removable heater/sensor module 2020, detector 2009 such as a fluorescent detection module, display screen or optionally combined display and user interface 2006 (e.g., a medical 50 grade touch sensitive liquid crystal display (LCD)). In some embodiments, lid 2010, detector 2009, and handle 2008 can be collectively referred to as slider module 2007. Additional components of apparatus 2000 may include one or more mechanical fixtures such as frame 2019 to hold the various 55 modules (e.g., the heater/sensor module 2020, and/or the slider module 2007) in alignment, and for providing structural rigidity. Detector module 2009 can be placed in rails to facilitate opening and placement of cartridge 2060 in the apparatus 2000, and to facilitate alignment of the optics upon 60 closing. Heater/sensor module 2020 can be also placed on rails for easy removal and insertion of the assembly.

Embodiments of apparatus 2000 also include software (e.g., for interfacing with users, conducting analysis and/or analyzing test results), firmware (e.g., for controlling the 65 hardware during tests on the cartridge 812), and one or more peripheral communication interfaces shown collectively as

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2031 for peripherals (e.g., communication ports such as USB/ Serial/Ethernet to connect to storage such as compact disc or hard disk, to connect input devices such as a bar code reader and/or a keyboard, to connect to other computers or storage via a network, and the like).

Control electronics 840, shown schematically in the block diagram in FIG. 8, can include one or more functions in various embodiments, for example for, main control 900, multiplexing 902, display control 904, detector control 906, and the like. The main control function may serve as the hub of control electronics 840 in apparatus 2000 and can manage communication and control of the various electronic functions. The main control function can also support electrical and communications interface 908 with a user or an output device such as a printer 920, as well as optional diagnostic and safety functions. In conjunction with main control function 900, multiplexer function 902 can control sensor data 914 and output current 916 to help control heater/sensor module 2020. The display control function 904 can control output to and, if applicable, interpret input from touch screen LCD 846, which can thereby provide a graphical interface to the user in certain embodiments. The detector function 906 can be implemented in control electronics 840 using typical control and processing circuitry to collect, digitize, filter, and/or transmit the data from a detector 2009 such as one or more fluorescence detection modules.

Microfluidic Cartridge

The present technology comprises a microfluidic cartridge that is configured to carry out an amplification, such as by PCR, of one or more polynucleotides from one or more samples. It is to be understood that, unless specifically made clear to the contrary, where the term PCR is used herein, any variant of PCR including but not limited to real-time and quantitative, and any other form of polynucleotide amplification is intended to be encompassed. The microfluidic cartridge need not be self-contained and can be designed so that it receives thermal energy from one or more heating elements present in an external apparatus with which the cartridge is in thermal communication. An exemplary such apparatus is further described herein; additional embodiments of such a system are found in U.S. patent application Ser. No. 11/940,310, entitled "Microfluidic Cartridge and Method of Making Same", and filed on even date herewith, the specification of which is incorporated herein by reference.

By cartridge is meant a unit that may be disposable, or reusable in whole or in part, and that is configured to be used in conjunction with some other apparatus that has been suitably and complementarily configured to receive and operate on (such as deliver energy to) the cartridge.

By microfluidic, as used herein, is meant that volumes of sample, and/or reagent, and/or amplified polynucleotide are from about 0.1  $\mu$ l to about 999  $\mu$ l, such as from 1-100  $\mu$ l, or from 2-25  $\mu$ l. Similarly, as applied to a cartridge, the term microfluidic means that various components and channels of the cartridge, as further described herein, are configured to accept, and/or retain, and/or facilitate passage of microfluidic volumes of sample, reagent, or amplified polynucleotide. Certain embodiments herein can also function with nanoliter volumes (in the range of 10-500 nanoliters, such as 100 nanoliters).

One aspect of the present technology relates to a microfluidic cartridge having two or more sample lanes arranged so that analyses can be carried out in two or more of the lanes in parallel, for example simultaneously, and wherein each lane is independently associated with a given sample.

A sample lane is an independently controllable set of elements by which a sample can be analyzed, according to

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methods described herein as well as others known in the art. A sample lane comprises at least a sample inlet, and a microfluidic network having one or more microfluidic components, as further described herein.

In various embodiments, a sample lane can include a sample inlet port or valve, and a microfluidic network that comprises, in fluidic communication one or more components selected from the group consisting of: at least one thermally actuated valve, a bubble removal vent, at least one thermally actuated pump, a gate, mixing channel, positioning element, microreactor, a downstream thermally actuated valve, and a PCR reaction chamber. The sample inlet valve can be configured to accept a sample at a pressure differential compared to ambient pressure of between about 70 and 100 kilopascals.

The cartridge can therefore include a plurality of microfluidic networks, each network having various components, and each network configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined.

A multi-lane cartridge is configured to accept a number of samples in series or in parallel, simultaneously or consecutively, in particular embodiments 12 samples, wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain 25 one or more polynucleotides in a form suitable for amplification. The polynucleotides in question may be the same as, or different from one another, in different samples and hence in different lanes of the cartridge. The cartridge typically processes each sample by increasing the concentration of a polynucleotide to be determined and/or by reducing the concentration of inhibitors relative to the concentration of polynucleotide to be determined.

The multi-lane cartridge comprises at least a first sample lane having a first microfluidic network and a second lane 35 having a second microfluidic network, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network is configured to amplify polynucleotides in the first sample, and wherein the second microfluidic 40 network is configured to amplify polynucleotides in the second sample.

In various embodiments, the microfluidic network can be configured to couple heat from an external heat source to a sample mixture comprising PCR reagent and neutralized 45 polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

At least the external heat source may operate under control of a computer processor, configured to execute computer 50 readable instructions for operating one or more components of each sample lane, independently of one another, and for receiving signals from a detector that measures fluorescence from one or more of the PCR reaction chambers.

For example, FIG. 9 shows a plan view of a microfluidic 55 cartridge 100 containing twelve independent sample lanes 101 capable of simultaneous or successive processing. The microfluidic network in each lane is typically configured to carry out amplification, such as by PCR, on a PCR-ready sample, such as one containing nucleic acid extracted from a sample using other methods as further described herein. A PCR-ready sample is thus typically a mixture comprising the PCR reagents and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide 65 sample. For example, a PCR-ready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive

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control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence. Exemplary probes are further described herein. Typically, the microfluidic network is configured to couple heat from an external heat source with the mixture comprising the PCR reagent and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

In various embodiments, the PCR reagent mixture can include a positive control plasmid and a plasmid fluorogenic hybridization probe selective for at least a portion of the plasmid, and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe.

In various embodiments, the microfluidic cartridge can accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide. Each lane of a multi-lane cartridge as described herein can perform two reactions when used in conjunction with two fluorescence detection systems per lane. A variety of combinations of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two other lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

FIG. 10A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. FIG. 10B shows a close-up view of a portion of the cartridge 200 of FIG. 10A illustrating various representative components. The cartridge 200 may be referred to as a multi-lane PCR cartridge with dedicated sample inlets 202. For example sample inlet 202 is configured to accept a liquid transfer member (not shown) such as a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown in FIGS. 10A, 10B, wherein one inlet operates in conjunction with a single sample lane. Various components of microfluidic circuitry in each lane are also visible. For example, microvalves 204, and 206, and hydrophobic vents 208 for removing air bubbles, are parts of microfluidic circuitry in a given lane. Also shown is an ultrafast PCR reactor 210, which, as further described herein, is a microfluidic channel in a given sample lane that is long enough to permit PCR to amplify polynucleotides present in a sample. Above each PCR reactor 210 is a window 212 that permits detection of fluorescence from a fluorescent substance in PCR reactor 210 when a detector is situated above window 212. It is to be understood that other configurations of windows are possible including, but not limited to, a single window that straddles each PCR reactor across the width of cartridge 200.

In preferred embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is customarily used in the art. Advantageously, then, such a cartridge may be used with plate handlers used elsewhere in the art.

The sample inlets of adjacent lanes are reasonably spaced apart from one another to prevent any contamination of one sample inlet from another sample when a user introduces a sample into any one cartridge. In an embodiment, the sample

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inlets are configured so as to prevent subsequent inadvertent introduction of sample into a given lane after a sample has already been introduced into that lane. In certain embodiments, the multi-sample cartridge is designed so that a spacing between the centroids of sample inlets is 9 mm, which is an industry-recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge that accept samples from PCR tubes, as further described herein, is 9 mm. The inlet holes can be manufactured conical in shape with an appropriate conical angle so that industry-standard pipette tips  $(2 \,\mu l, 20 \,\mu l, 200 \,\mu l, volumes, etc.)$  fit snugly therein. The cartridge herein may be adapted to suit other, later-arising, industry standards not otherwise described herein, as would be understood by one of ordinary skill in the art.

In one embodiment, an exemplary microfluidic cartridge has 12 sample lanes. The inlet ports in this embodiment have a 6 mm spacing, so that, when used in conjunction with an automated sample loader having 4 heads, spaced equidistantly at 18 mm apart, the inlets can be loaded in three batches of four inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from one side of the cartridge to the other as shown.

A microfluidic cartridge as used herein may be constructed 25 from a number of layers. Accordingly, one aspect of the present technology relates to a microfluidic cartridge that comprises a first, second, third, fourth, and fifth layers wherein one or more layers define a plurality of microfluidic networks, each network having various components configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined. In various embodiments, one or more such layers are optional.

FIGS. 11A-C show various views of a layer structure of an 35 exemplary microfluidic cartridge comprising a number of layers, as further described herein. FIG. 11A shows an exploded view; FIG. 11B shows a perspective view; and FIG. 11C shows a cross-sectional view of a sample lane in the exemplary cartridge. Referring to FIGS. 11A-C, an exemplary microfluidic cartridge 400 includes first 420, second 422, third 424, fourth 426, and fifth layers in two non-contiguous parts 428, 430 (as shown) that enclose a microfluidic network having various components configured to process multiple samples in parallel that include one or more polynucleotides to be determined.

Microfluidic cartridge 400 can be fabricated as desired. The cartridge can include a microfluidic substrate layer 424, typically injection molded out of a plastic, such as a zeonor plastic (cyclic olefin polymer), having a PCR channel and 50 valve channels on a first side and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a second side (disposed toward hydrophobic vent membrane 426). It is advantageous that all the microfluidic network defining structures, such as PCR reactors, valves, inlet 55 holes, and air vents, are defined on the same single substrate 424. This attribute facilitates manufacture and assembly of the cartridge. Additionally, the material from which this substrate is formed is rigid or non-deformable, non-venting to air and other gases, and has a low autofluorescence to facilitate 60 detection of polynucleotides during an amplification reaction performed in the microfluidic circuitry defined therein. Rigidity is advantageous because it facilitates effective and uniform contact with a heat unit as further described herein. Use of a non-venting material is also advantageous because it 65 reduces the likelihood that the concentration of various species in liquid form will change during analysis. Use of a

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material having low auto-fluorescence is also important so that background fluorescence does not detract from measurement of fluorescence from the analyte of interest.

The cartridge can further include, disposed on top of the substrate 424, an oleophobic/hydrophobic vent membrane layer 426 of a porous material, such as 0.2 to 1.0 micron pore-size membrane of modified polytetrafluorethylene, the membrane being typically between about 25 and about 100 microns thick, and configured to cover the vent channels of microfluidic substrate 424, and attached thereto using, for example, heat bonding.

Typically, the microfluidic cartridge further includes a layer 428, 430 of polypropylene or other plastic label with pressure sensitive adhesive (typically between about 50 and 150 microns thick) configured to seal the wax loading holes of the valves in substrate 424, trap air used for valve actuation, and serve as a location for operator markings. In FIG. 4A, this layer is shown in two separate pieces, 428, 430, though it would be understood by one of ordinary skill in the art that a single piece layer would be appropriate.

In various embodiments, the label is a computer-readable label. For example, the label can include a bar code, a radio frequency tag or one or more computer-readable characters. The label can be formed of a mechanically compliant material. For example, the mechanically compliant material of the label can have a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100. The label can be positioned such that it can be read by a sample identification verifier as further described herein.

The cartridge can further include a heat sealable laminate layer 422 (typically between about 100 and about 125 microns thick) attached to the bottom surface of the microfluidic substrate 424 using, for example, heat bonding. This layer serves to seal the PCR channels and vent channels in substrate 424. The cartridge can further include a thermal interface material layer 420 (typically about 125 microns thick), attached to the bottom of the heat sealable laminate layer using, for example, pressure sensitive adhesive. The layer 420 can be compressible and have a higher thermal conductivity than common plastics, thereby serving to transfer heat across the laminate more efficiently. Typically, however, layer 420 is not present.

The application of pressure to contact the cartridge to the heater of an instrument that receives the cartridge generally assists in achieving better thermal contact between the heater and the heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was only partially filled with liquid and the air entrapped therein would be thermally expanded during thermocycling.

In use, cartridge 400 is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, actuators, and processing region 410) of the device. Exemplary such heater arrays are further described herein. Additional embodiments of heater arrays are described in U.S. patent application Ser. No. 11/940,315, entitled "Heater Unit for Microfluidic Diagnostic System" and filed on even date herewith, the specification of which is incorporated herein by reference in its entirety. In some embodiments, the heat sources are controlled by a computer processor and actuated according to a desired protocol. Processors configured to operate microfluidic devices are described in, e.g., U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference.

In various embodiments, during transport and storage, the microfluidic cartridge can be further surrounded by a sealed

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pouch. The microfluidic cartridge can be sealed in the pouch with an inert gas. The microfluidic cartridge can be disposable for example after one or more of its sample lanes have been used.

**Highly Multiplexed Embodiments** 

Embodiments of the cartridge described herein may be constructed that have high-density microfluidic circuitry on a single cartridge that thereby permit processing of multiple samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 20, 24, 36, 40, 48, 50, 60, 64, 72, 80, 84, 96, and 100, but it would be understood that still other numbers are consistent with the apparatus and cartridge herein, where deemed convenient and practical.

Accordingly, different configurations of lanes, sample inlets, and associated heater networks than those explicitly depicted in the FIGs and examples that can facilitate processing such numbers of samples on a single cartridge are within the scope of the instant disclosure. Similarly, alternative configurations of detectors and heating elements for use in conjunction with such a highly multiplexed cartridge are also within the scope of the description herein.

It is also to be understood that the microfluidic cartridges described herein are not to be limited to rectangular shapes, 25 but can include cartridges having circular, elliptical, triangular, rhombohedral, square, and other shapes. Such shapes may also be adapted to include some irregularity, such as a cut-out, to facilitate placement in a complementary apparatus as further described herein.

In an exemplary embodiment, a highly multiplexed cartridge has 48 sample lanes, and permits independent control of each valve in each lane by suitably configured heater circuitry, with 2 banks of thermocycling protocols per lane, as shown in FIG. 12. In the embodiment in FIG. 12, the heaters (shown superimposed on the lanes) are arranged in three arrays 502, 504, with 506, and 508. The heaters are themselves disposed within one or more substrates. Heater arrays 502, 508 in two separate glass regions only apply heat to 40 valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and passed to the PCR reaction chambers independently of one 45 another. The PCR heaters 504, 506 are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. It is preferable for the PCR heaters to be arranged in 2 banks (the heater arrays 506 on the left and right 508 are not in electrical communication with one another), thereby permitting a separate degree of sample control.

FIG. 13 shows a representative 48-sample cartridge 600 compatible with the heater arrays of FIG. 12, and having a configuration of inlets 602 different to that depicted o other cartridges herein. The inlet configuration is exemplary and has been designed to maximize efficiency of space usage on the cartridge. The inlet configuration can be compatible with an automatic pipetting machine that has dispensing heads situated at a 9 mm spacing. For example, such a machine having 4 heads can load 4 inlets at once, in 12 discrete steps, for the cartridge of FIG. 13. Other configurations of inlets though not explicitly described or depicted are compatible with the technology described herein.

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FIG. 14 shows, in close up, an exemplary spacing of valves 702, channels 704, and vents 796, in adjacent lanes 708 of a multi-sample microfluidic cartridge for example as shown in FIG. 13.

FIGS. 15 and 16 show close-ups of, respectively, heater arrays 804 compatible with, and inlets 902 on, the exemplary cartridge shown in FIG. 14.

FIGS. 17A and 17B show various views of an embodiment of a radially-configured highly-multiplexed cartridge, having a number of inlets 1002, microfluidic lanes 1004, valves 1005, and PCR reaction chambers 1006. FIG. 17C shows an array of heater elements 1008 compatible with the cartridge layout of FIG. 17A.

The various embodiments shown in FIGS. **12-17**C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the other specific examples described herein.

During the design and manufacture of highly multiplexed cartridges, photolithographic processing steps such as etching, hole drilling/photo-chemical drilling/sand-blasting/ion-milling processes should be optimized to give well defined holes and microchannel pattern. Proper distances between channels should be identified and maintained to obtain good bonding between the microchannel substate and the heat conducting substrate layer. In particular, it is desirable that minimal distances are maintained between pairs of adjacent microchannels to promote, reliable bonding of the laminate in between the channels.

The fabrication by injection molding of these complicated microfluidic structures having multiple channels and multiple inlet holes entails proper consideration of dimensional repeatability of these structures over multiple shots from the injection molding master pattern. Proper consideration is also attached to the placement of ejector pins to push out the structure from the mold without causing warp, bend or stretching of it. For example, impression of the ejector pins on the microfluidic substrate should not sink into the substrate thereby preventing planarity of the surface of the cartridge. The accurate placement of various inlet holes (such as sample inlet holes, valve inlet holes and vent holes) relative to adjacent microfluidic channels is also important because the presence of these holes can cause knit-lines to form that might cause unintended leak from a hole to a microchannel. Highly multiplexed microfluidic substrates may be fabricated in other materials such as glass, silicon.

The size of the substrate relative to the number of holes is also factor during fabrication because it is easy to make a substrate having just a simple microfluidic network with a few holes (maybe fewer than 10 holes) and a few microchannels, but making a substrate having over 24, or over 48, or over 72 holes, etc., is more difficult.

Microfluidic Networks

Particular components of exemplary microfluidic networks are further described herein.

Channels of a microfluidic network in a lane of cartridge typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

FIG. 18 shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. 10A and 10B. It would be understood by one skilled in the art that other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In operation of the cartridge, in sequence, sample is introduced through liquid

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inlet 202, optionally flows into a bubble removal vent channel 208 (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel 216. Typically, when using a robotic dispenser of liquid sample, the volume is dispensed accurately enough that 5 formation of bubbles is not a significant problem, and the presence of vent channel 208 is not necessary. Thus, in certain embodiments, the bubble removal vent channel 208 is not present and sample flows directly into channel 216. Throughout the operation of cartridge 200, the fluid is manipulated as 10 a microdroplet (not shown in the FIGs). Valves 204 and 206 are initially both open, so that a microdroplet of samplecontaining fluid can be pumped into PCR reactor channel 210 from inlet hole 202 under influence of force from the sample injection operation. Upon initiating of processing, the detec- 15 tor present on top of the PCR reactor 210 checks for the presence of liquid in the PCR channel, and then valves 204 and 206 are closed to isolate the PCR reaction mix from the outside. In one embodiment, the checking of the presence of liquid in the PCR channel is by measuring the heat ramp rate. 20 such as by one or more temperature sensors in the heating unit. A channel with liquid absent will heat up faster than one in which, e.g., a sample, is present.

Both valves **204** and **206** are closed prior to thermocycling to prevent or reduce any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor. End vent **214** is configured to prevent a user from introducing an excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample volumes as small as an amount to fill the region from the bubble removal vent (if present) to the middle of the microreactor, or up to valve **204** or beyond valve **204**. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction.

The reactor 210 is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein, and according to amplification protocols known to those of ordinary skill 40 in the art. The inside walls of the channel in the PCR reactor are typically made very smooth and polished to a shiny finish (for example, using a polish selected from SPI A1, SPI A2, SPI A3, SPI B1, or SPI B2) during manufacture. This is in order to minimize any microscopic quantities of air trapped in 45 the surface of the PCR channel, which would causing bubbling during the thermocycling steps. The presence of bubbles especially in the detection region of the PCR channel could also cause a false or inaccurate reading while monitoring progress of the PCR. Additionally, the PCR channel can 50 be made shallow such that the temperature gradient across the depth of the channel is minimized.

The region of the cartridge 212 above PCR reactor 210 is a thinned down section to reduce thermal mass and autofluorescence from plastic in the cartridge. It permits a detector to 55 more reliably monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. Exemplary probes are further described herein. The region 212 can be made of thinner material than the rest of the cartridge so as to permit the PCR channel to be 60 more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denaturing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence.

After PCR has been carried out on a sample, and presence 65 or absence of a polynucleotide of interest has been determined, it is preferred that the amplified sample remains in the

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cartridge and that the cartridge is either used again (if one or more lanes remain unused), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the laminate of the cartridge, and recover an amount—typically about 1.5 microliter—of PCR product. The user may also place the individual PCR lane on a special narrow heated plate, maintained at a temperature to melt the wax in the valve, and then aspirate the reacted sample from the inlet hole of that PCR lane

In various embodiments, the microfluidic network can optionally include at least one reservoir configured to contain waste.

Table 1 outlines typical volumes, pumping pressures, and operation times associated with various components of a microfluidic cartridge described herein.

TABLE 1

Operation	Pumping Press	Displacement sure Volume	Time of Operation
Moving valve w plugs	ax ~1-2 psi	<1 μl	5-15 seconds
Operation	Pump Used	Pump Design	Pump Actuation
Moving valve wax plugs	Thermopneumatic pump	1 μl of trapped air	Heat trapped air to ~70-90 C.

Valves

A valve (sometimes referred to herein as a microvalve) is a component in communication with a channel, such that the valve has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Upon actuation of the valve, the valve transitions to a closed state that prevents material from passing along the channel from one side of the valve to the other. For example, in one embodiment, a valve can include a mass of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage when the valve is closed. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The TRS can also be a blend of variety of materials, such as an emulsion of thermoelastic polymer blended with air microbubbles (to enable higher thermal expansion, as well as reversible expansion and contraction), polymer blended with expancel material (offering higher thermal expansion), polymer blended with heat conducting microspheres (offering faster heat conduction and hence, faster melting profiles), or a polymer blended with magnetic microspheres (to permit magnetic actuation of the melted thermoresponsive material).

Generally, for such a valve, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less). Typically, a chamber is in gaseous communication with the mass of TRS. The valve is in communication with a source of heat that can be selectively applied to the chamber of air and to the TRS. Upon heating gas (e.g., air) in the chamber and heating the mass of TRS to the second temperature, gas pressure within

the chamber due to expansion of the volume of gas, forces the mass to move into the channel, thereby obstructing material from passing therealong.

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An exemplary valve is shown in FIG. 19A. The valve of FIG. 19A has two chambers of air 1203, 1205 in contact with, 5 respectively, each of two channels 1207, 1208 containing TRS. The air chambers also serve as loading ports for TRS during manufacture of the valve, as further described herein. In order to make the valve sealing very robust and reliable, the flow channel 1201 (along which, e.g., sample passes) at the 10 valve junction is made narrow (typically 150 µm wide, and 150 µm deep or narrower), and the constricted portion of the flow channel is made at least 0.5 or 1 mm long such that the TRS seals up a long narrow channel thereby reducing any leakage through the walls of the channel. In the case of a bad 15 seal, there may be leakage of fluid around walls of channel, past the TRS, when the valve is in the closed state. In order to minimize this, the flow channel is narrowed and elongated as much as possible. In order to accommodate such a length of channel on a cartridge where space may be at a premium, the 20 flow channel can incorporate one or more curves 1209 as shown in FIG. 19A. The valve operates by heating air in the TRS-loading port, which forces the TRS forwards into the flow-channel in a manner so that it does not come back to its original position. In this way, both air and TRS are heated 25 during operation.

In various other embodiments, a valve for use with a microfluidic network in a microfluidic cartridge herein can be a bent valve as shown in FIG. 19B. Such a configuration reduces the footprint of the valve and hence reduces cost per 30 part for highly dense microfluidic cartridges. A single valve loading hole 1211 is positioned in the center, that serves as an inlet for thermally responsive substance. The leftmost vent 1213 can be configured to be an inlet for, e.g., sample, and the rightmost vent 1215 acts as an exit for, e.g., air. This configuration can be used as a prototype for testing such attributes as valve and channel geometry and materials.

In various other embodiments, a valve for use with a microfluidic network can include a curved valve as shown in FIG. 19C, in order to reduce the effective cross-section of the 40 valve, thereby enabling manufacture of cheaper dense microfluidic devices. Such a valve can function with a single valve loading hole and air chamber 1221 instead of a pair as shown in FIG. 19A.

FIG. 19D shows an exemplary gate as may optionally be used in a microfluidic network herein. A gate can be a component that can have a closed state that does not allow material to pass along a channel from a position on one side of the gate to another side of the gate, and an open state that does allow material to pass along a channel from a position on one side of the gate to another side of the gate. Actuation of an open gate can transition the gate to a closed state in which material is not permitted to pass from one side of the gate (e.g., upstream of the gate) to the other side of the gate (e.g., 55 downstream of the gate). Upon actuation, a closed gate can transition to an open state in which material is permitted to pass from one side of the gate (e.g., upstream of the gate) to the other side of the gate (e.g., downstream of the gate).

In various embodiments, a microfluidic network can 60 include a narrow gate **380** as shown in FIG. **19D** where a gate loading channel **382** used for loading wax from a wax loading hole **384** to a gate junction **386** can be narrower (e.g., approximately 150  $\mu$ m wide and 100 microns deep). An upstream channel **388** as well as a downstream channel **390** of the gate 65 junction **386** can be made wide (e.g., 500  $\mu$ m) and deep (e.g., 500  $\mu$ m) to help ensure the wax stops at the gate junction **386**.

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The amount of gate material melted and moved out of the gate junction 386 may be minimized for optimal gate 380 opening. As an off-cartridge heater may be used to melt the thermally responsive substance in gate 380, a misalignment of the heater could cause the wax in the gate loading channel 382 to be melted as well. Therefore, narrowing the dimension of the loading channel may increase reliability of gate opening. In the case of excessive amounts of wax melted at the gate junction 386 and gate loading channel 382, the increased cross-sectional area of the downstream channel 390 adjacent to the gate junction 386 can prevent wax from clogging the downstream channel 390 during gate 380 opening. The dimensions of the upstream channel 388 at the gate junction 386 can be made similar to the downstream channel 390 to ensure correct wax loading during gate fabrication.

In various embodiments, the gate can be configured to minimize the effective area or footprint of the gate within the network and thus bent gate configurations, although not shown herein are consistent with the foregoing description. Vents

In various embodiments, the microfluidic network can include at least one hydrophobic vent in addition to an end vent. A vent is a general outlet (hole) that may or may not be covered with a hydrophobic membrane. An exit hole is an example of a vent that need not be covered by a membrane.

A hydrophobic vent (e.g., a vent in FIG. 20) is a structure that permits gas to exit a channel while limiting (e.g., preventing) quantities of liquid from exiting the channel. Typically, hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from GE Osmonics, Minnetonka, Minn.) that defines a wall of the channel. As described elsewhere herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microfluidic network.

The hydrophobic vents of the present technology are preferably constructed so that the amount of air that escapes through them is maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane 1303 of large surface area and a shallow cross section of the microchannel below the vent surface.

Hydrophobic vents are useful for bubble removal and typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel **1305** (see FIG. **13**). The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less).

The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of the depth of the channel upstream 1301 and downstream (not shown) of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns. Other dimensions are consistent with the description herein.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns, and the width of the channel

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upstream and downstream from the vent is about 250 microns. Other dimensions are consistent with the description berein

The vent in FIG. **20** is shown in a linear configuration though it would be understood that it need not be so. A bent, 5 kinked, curved, S-shaped, V-shaped, or U-shaped (as in item **208** FIG. **11**) vent is also consistent with the manner of construction and operation described herein.

Use of Cutaways in Cartridge and Substrate To Improve Rate of Cooling During PCR Cycling

During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved efficiency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the heating substrate and/or the cartridge, as shown in FIG. 21.

One way to achieve rapid cooling is to cutaway portions of the microfluidic cartridge substrate, as shown in FIG. 22A. The upper panel of FIG. 22A is a cross-section of an exemplary microfluidic cartridge taken along the dashed line A-A' 20 as marked on the lower panel of FIG. 22A. PCR reaction chamber 1601, and representative heaters 1603 are shown. Also shown are two cutaway portions, one of which labeled 1601, that are situated alongside the heaters that are positioned along the long side of the PCR reaction chamber. 25 Cutaway portions such as 1601 reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction chamber. Other configurations of cutouts, such as in 30 shape, position, and number, are consistent with the present technology.

Another way to achieve rapid cooling is to cutaway portions of the heater substrate, as shown in FIG. 22B. The lower panel of FIG. 22B is a cross-section of an exemplary microf- 35 luidic cartridge and heater substrate taken along the dashed line A-A' as marked on the upper panel of FIG. 22B. PCR reaction chamber 901, and representative heaters 1003 are shown. Also shown are four cutaway portions, one of which labeled 1205, that are situated alongside the heaters that are 40 situated along the long side of the PCR reaction chamber. Cutaway portions such as 1205 reduce the thermal mass of the heater substrate, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the 45 PCR reaction chamber. Four separate cutaway portions are shown in FIG. 22A so that control circuitry to the various heaters is not disrupted. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology. These cutouts may be created by a 50 method selected from: selective etching using wet etching processes, deep reactive ion etching, selective etching using CO<sub>2</sub> laser or femtosecond laser (to prevent surface cracks or stress near the surface), selective mechanical drilling, selective ultrasonic drilling, or selective abrasive particle blasting. 55 Care has to be taken to maintain mechanically intergrity of the heater while reducing as much material as possible.

FIG. 22C shows a combination of cutouts and use of ambient air cooling to increase the cooling rate during the cooling stage of thermocycling. A substantial amount of cooling happens by convective loss from the bottom surface of the heater surface to ambient air. The driving force for this convective loss is the differential in temperatures between the glass surface and the air temperature. By decreasing the ambient air temperature by use of, for example, a peltier cooler, the rate of cooling can be increased. The convective heat loss may also be increased by keeping the air at a velocity higher than zero.

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An example of thermal cycling performance in a PCR reaction chamber obtained with a configuration as described herein, is shown in FIG. 23 for a protocol that is set to heat up the reaction mixture to 92° C., and maintain the temperature for 1 second, then cool to 62° C., and stay for 10 seconds. The cycle time shown is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C. To minimize the overall time required for a PCR effective to produce detectable quantities of amplified material, it is important to minimize the time required for each cycle. Cycle times in the range 15-30 s, such as 18-25 s, and 20-22 s, are desirable. In general, an average PCR cycle time of 25 seconds as well as cycle times as low as 20 seconds are typical with the technology described herein. Using reaction volumes less than a microliter (such as a few hundred nanoliters or less) permits use of an associated smaller PCR chamber, and enables cycle times as low as 15 seconds. An average cycle time of 25 seconds and as low as 20 seconds can be achieved by technology described herein, even without any forced cooling or implementing any thermal mass reductions described elsewhere herein.

Manufacturing Process for Cartridge

FIG. 24 shows a flow-chart 1800 for an embodiment of an assembly process for an exemplary cartridge as shown in FIG. 11A herein. It would be understood by one of ordinary skill in the art, both that various steps may be performed in a different order from the order set forth in FIG. 24, and additionally that any given step may be carried out by alternative methods to those described in the figure. It would also be understood that, where separate serial steps are illustrated for carrying out two or more functions, such functions may be performed synchronously and combined into single steps and remain consistent with the overall process described herein.

At **1802**, a laminate layer is applied to a microfluidic substrate that has previously been engineered, for example by injection molding, to have a microfluidic network constructed in it; edges are trimmed from the laminate where they spill over the bounds of the substrate.

At **1804**, wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate. An exemplary process for carrying this out is further described herein

At 1806, the substrate is inspected to ensure that wax from step 1804 is loaded properly and that the laminate from step 1802 adheres properly to it. If a substrate does not satisfy either or both of these tests, it is usually discarded. If substrates repeatedly fail either or both of these tests, then the wax dispensing, or laminate application steps, as applicable, are reviewed.

At 1808, a hydrophobic vent membrane is applied to, and heat bonded to, the top of the microfluidic substrate covering at least the one or more vent holes, and on the opposite face of the substrate from the laminate. Edges of the membrane that are in excess of the boundary of the substrate are trimmed.

At 1810, the assembly is inspected to ensure that the hydrophobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is discarded, and, in the case of repeated discard events, the foregoing process step 1808 is reviewed.

At 1812, optionally, a thermally conductive pad layer is applied to the bottom laminate of the cartridge.

At 1814, two label strips are applied to the top of the microfluidic substrate, one to cover the valves, and a second

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to protect the vent membranes. It would be understood that a single label strip may be devised to fulfill both of these roles.

At **1816**, additional labels are printed or applied to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Preferably one or more of these 5 labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

Optionally, at **1818**, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked, and cartridges packed into groups, such as groups of 25, or groups of 10, or groups of 20, or groups of 48 or 50. Preferably the packaging is via an inert and/or moisture-free medium. Wax Loading in Valves

In general, a valve as shown in, e.g., FIGS. 25A-C, is constructed by depositing a precisely controlled amount of a 15 TRS (such as wax) into a loading inlet machined in the microfluidic substrate. FIGS. 25A and 25B show how a combination of controlled hot drop dispensing into a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The top of FIG. 25A shows a plan view of a valve inlet 190 and loading channel 1902, connecting to a flow channel 1904. The lower portions of FIG. 25A show the progression of a dispensed wax droplet 1906 (having a volume of 75 nl±15 nl) through the inlet 1901 and 25 into the loading channel 1902.

To accomplish those steps, a heated dispenser head can be accurately positioned over the inlet hole of the microchannel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of 20%. 30 A suitable dispenser is also one that can deposit amounts smaller than 100 nl with a precision of +/-20%. The dispenser should also be capable of heating and maintaining the dispensing temperature of the TRS to be dispensed. For example, it may have a reservoir to hold the solution of TRS. 35 It is also desirable that the dispense head can have freedom of movement at least in a horizontal (x-y) plane so that it can easily move to various locations of a microfluidic substrate and dispense volumes of TRS into valve inlets at such locations without having to be re-set, repositioned manually, or 40 recalibrated in between each dispense operation.

The inlet hole of the microfluidic cartridge, or other microchannel device, is dimensioned in such a way that the droplet of 75 nl can be accurately propelled to the bottom of the inlet hole using, for example, compressed air, or in a manner 45 similar to an inkjet printing method. The microfluidic cartridge is maintained at a temperature above the melting point of the wax thereby permitting the wax to stay in a molten state immediately after it is dispensed. After the drop falls to the bottom of the inlet hole 1901, the molten wax is drawn into 50 the narrow channel by capillary action, as shown in the sequence of views in FIG. 25B. A shoulder between the inlet hole 1901 and the loading channel can facilitate motion of the TRS. The volume of the narrow section can be designed to be approximately equal to a maximum typical amount that is 55 dispensed into the inlet hole. The narrow section can also be designed so that even though the wax dispensed may vary considerably between a minimum and a maximum shot size, the wax always fills up to, and stops at, the microchannel junction 1907 because the T-junction provides a higher cross 60 section than that of the narrow section and thus reduces the capillary forces.

#### PCR Reagent Mixtures

In various embodiments, the sample for introduction into a lane of the microfluidic cartridge can include a PCR reagent 65 mixture comprising a polymerase enzyme and a plurality of nucleotides.

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In various embodiments, preparation of a PCR-ready sample for use with an apparatus and cartridge as described herein can include contacting a neutralized polynucleotide sample with a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid).

The PCR-ready sample can be prepared, for example, using the following steps: (1) collect sample in sample collection buffer, (2) transfer entire sample to lysis tube, mix, heat, and incubate for seven minutes, (3) place on magnetic rack, allow beads to separate, aspirate supernatant, (4) add  $100\,\mu$ l of Buffer 1, mix, place on magnetic rack, allow beads to separate, aspirate supernatant, (5) add  $10\,\mu$ l of Buffer 2, mix, place in high temperature heat block for 3 minutes, place on magnetic rack, allow beads to separate, transfer 5  $\mu$ l supernatant, and (6) Add 5  $\mu$ l of Buffer 3, transfer 1 to  $10\,\mu$ l of supernatant for PCR amplification and detection.

The PCR reagent mixture can be in the form of one or more lyophilized pellets and the steps by which the PCR-ready sample is prepared can involve reconstituting the PCR pellet by contacting it with liquid to create a PCR reagent mixture solution. In yet another embodiment, each of the PCR lanes may have dried down or lyophilized ASR reagents preloaded such that the user only needs to input prepared polynucleotide sample into the PCR. In another embodiment, the PCR lanes may have only the application-specific probes and primers pre-measured and pre-loaded, and the user inputs a sample mixed with the PCR reagents.

In various embodiments, the PCR-ready sample can include at least one probe that can be selective for a polynucleotide sequence, wherein the steps by which the PCR-ready sample is prepared involve contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the probe. The probe can be a fluorogenic hybridization probe. The fluorogenic hybridization probe can include a polynucleotide sequence coupled to a fluorescent reporter dye and a fluorescence quencher dye.

In various embodiments, the PCR-ready sample further includes a sample buffer.

In various embodiments, the PCR-ready sample includes at least one probe that is selective for a polynucleotide sequence, e.g., the polynucleotide sequence that is characteristic of a pathogen selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the PCR reagent mixture can further include a polymerase enzyme, a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism, for example any organism that employs deoxyribonucleic acid or ribonucleic acid polynucleotides. Thus, the probe can be selective for any organism. Suitable organisms include mammals (including humans), birds, reptiles, amphibians, fish, domesticated animals, wild animals, extinct organisms, bacteria, fungi, viruses, plants, and the like. The probe can also be selective for components of organisms that employ their own polynucleotides, for example mitochondria. In some embodiments, the probe is selective for microorganisms, for example, organisms used in food production (for example, yeasts employed in fermented products, molds or bacteria employed in cheeses, and the like) or pathogens (e.g., of humans, domesticated or wild mammals, domesticated or wild birds, and the like). In some embodiments, the probe is

27 selective for organisms selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi,

protozoa, and viruses.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism 5 selected from the group consisting of Staphylococcus spp., e.g., S. epidermidis, S. aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Staphylococcus; Streptococcus(e.g., α, β or γ-hemolytic, Group A, B, C, D or G) such as S. pyogenes, S. agalactiae; E. faecalis, E. durans, and E. faecium (formerly S. faecalis, S. durans, S. faecium); nonenterococcal group D streptococci, e.g., S. bovis and S. equines; Streptococci viridans, e.g., S. mutans, S. sanguis, S. salivarius, S. mitior, A. milleri, S. constellatus, S. intermedius, and S. anginosus; S. iniae; S. pneumoniae; Neis- 15 seria, e.g., N. meningitides, N. gonorrhoeae, saprophytic Neisseria sp; Erysipelothrix, e.g., E. rhusiopathiae; Listeria spp., e.g., L. monocytogenes, rarely L. ivanovii and L. seeligeri; Bacillus, e.g., B. anthracis, B. cereus, B. subtilis, B. subtilus niger, B. thuringiensis; Nocardia asteroids; 20 Legionella, e.g., L. pneumonophilia, Pneumocystis, e.g., P. carinii; Enterobacteriaceae such as Salmonella, Shigella, Escherichia (e.g., E. coli, E. coliO157:H7); Klebsiella, Enterobacter, Serratia, Proteus, Morganella, Providencia, Yersinia, and the like, e.g., Salmonella, e.g., S. typhi S. 25 paratyphi A, B (S. schottmuelleri), and C(S. hirschfeldii), S. dublin S. choleraesuis, S. enteritidis, S. typhimurium, S. heidelberg, S. newport, S. infantis, S. agona, S. montevideo, and S. saint-paul; Shigella e.g., subgroups: A, B, C, and D, such as S. flexneri, S. sonnei, S. boydii, S. dysenteriae; Pro- 30 teus (P. mirabilis, P. vulgaris, and P. myxofaciens), Morganella (M. morganii); Providencia (P. rettgeri, P. alcalifaciens, and P. stuartii); Yersinia, e.g., Y. pestis, Y. enterocolitica; Haemophilus, e.g., H. influenzae, H. parainfluenzae H. aphrophilus, H. ducreyi; Brucella, e.g., B. abor- 35 tus, B. melitensis, B. suis, B. canis; Francisella, e.g., F. tularensis; Pseudomonas, e.g., P. aeruginosa, P. paucimobilis, P. putida, P. fluorescens, P. acidovorans, Burkholderia (Pseudomonas) pseudomallei, Burkholderia Burkholderia cepacia and Stenotrophomonas maltophilia; 40 Campylobacter, e.g., C. fetus fetus, C. jejuni, C. pylori (Helicobacter pylori); Vibrio, e.g., V. cholerae, V. parahaemolyticus, V. mimicus, V. alginolyticus, V. hollisae, V. vulnificus, and the nonagglutinable vibrios; Clostridia, e.g., C. perfringens, C. tetani, C. difficile, C. botulinum; Actinomyces, e.g., A. 45 israelii; Bacteroides, e.g., B. fragilis, B. thetaiotaomicron, B. distasonis, B. vulgatus, B. ovatus, B. caccae, and B. merdae: Prevotella, e.g., P. melaminogenica; genus Fusobacterium; Treponema, e.g. T. pallidum subspecies endemicum, T. pallidum subspecies pertenue, T. carateum, and T. pallidum sub- 50 species pallidum; genus Borrelia, e.g., B burgdorferi; genus Leptospira; Streptobacillus, e.g., S. moniliformis; Spirillum, e.g., S. minus; Mycobacterium, e.g., M. tuberculosis, M. bovis, M. africanum, M. avium M. intracellulare, M. kansasii, M. xenopi, M. marinum, M. ulcerans, the M. fortuitum com- 55 plex (M. fortuitum and M. chelonei), M. leprae, M. asiaticum, M. chelonei subsp. abscessus, M. fallax, M. fortuitum, M. malmoense, M. shimoidei, M. simiae, M. szulgai, M. xenopi; Mycoplasma, e.g., M. hominis, M. orale, M. salivarium, M. fermentans, M. pneumoniae, M. bovis, M. tuberculosis, M. 60 avium, M. leprae; Mycoplasma, e.g., M. genitalium; Ureaplasma, e.g., U. urealyticum; Trichomonas, e.g., T. vaginalis; Cryptococcus, e.g., C. neoformans; Histoplasma, e.g., H. capsulatum; Candida, e.g., C. albicans; Aspergillus sp; Coccidioides, e.g., C. immitis; Blastomyces, e.g. B. dermatitidis; 65 Paracoccidioides, e.g., P. brasiliensis; Penicillium, e.g., P. marneffei; Sporothrix, e.g., S. schenckii; Rhizopus, Rhizomu28

cor, Absidia, and Basidiobolus; diseases caused by Bipolaris, Cladophialophora, Cladosporium, Drechslera, Exophiala, Fonsecaea, Phialophora, Xylohypha, Ochroconis, Rhinocladiella, Scolecobasidium, and Wangiella; Trichosporon, e.g., T. beigelii; Blastoschizomyces, e.g., B. capitatus; Plasmodium, e.g., P. falciparum, P. vivax, P. ovale, and P. malariae; Babesia sp; protozoa of the genus Trypanosoma, e.g., T. cruzi; Leishmania, e.g., L. donovani, L. major L. tropica, L. mexicana, L. braziliensis, L. viannia braziliensis; Toxoplasma, e.g., T. gondii; Amoebas of the genera Naegleria or Acanthamoeba; Entamoeba histolytica; Giardia lamblia; genus Cryptosporidium, e.g., C. parvum; Isospora belli; Cyclospora cayetanensis; Ascaris lumbricoides; Trichuris trichiura; Ancylostoma duodenale or Necator americanus; Strongyloides stercoralis Toxocara, e.g., T. canis, T. cati; Baylisascaris, e.g., B. procyonis; Trichinella, e.g., T. spiralis; Dracunculus, e.g., D. medinensis; genus Filarioidea; Wuchereria bancrofti; Brugia, e.g., B. malayi, or B. timori; Onchocerca volvulus; Loa loa; Dirofilaria immitis; genus Schistosoma, e.g., S. japonicum, S. mansoni, S. mekongi, S. intercalatum, S. haematobium; Paragonimus, e.g., P. Westermani, P. Skriabini; Clonorchis sinensis; Fasciola hepatica; Opisthorchis sp; Fasciolopsis buski; Diphyllobothrium latum; Taenia, e.g., T. saginata, T. solium; Echinococcus, e.g., E. granulosus, E. multilocularis; Picornaviruses, rhinoviruses echoviruses, coxsackieviruses, influenza virus; paramyxoviruses, e.g., types 1, 2, 3, and 4; adnoviruses; Herpesviruses, e.g., HSV-1 and HSV-2; varicella-zoster virus; human T-lymphotrophic virus (type I and type II); Arboviruses and Arenaviruses; Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae; Flavivirus; Hantavirus; Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]); Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo]); Smallpox (variola); retroviruses e.g., human immunodeficiency viruses 1 and 2; human papillomavirus [HPV] types 6, 11, 16, 18, 31, 33, and 35.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organisms selected from the group consisting of *Pseudomonas* aeruginosa, Proteus mirabilis, Klebsiella oxytoca, Klebsiella pneumoniae, Escherichia coli, Acinetobacter Baumannii, Serratia marcescens, Enterobacter aerogenes, Enterococcus faecium, vancomycin-resistant enterococcus (VRE), Staphylococcus aureus, methecillin-resistant Staphylococcus aureus(MRSA), Streptococcus viridans, Listeria monocytogenes, Enterococcus spp., Streptococcus Group B, Streptococcus Group C, Streptococcus Group G, Streptococcus Group F, Enterococcus faecalis, Streptococcus pneumoniae, Staphylococcus epidermidis, Gardenerella vaginalis, Micrococcus sps., Haemophilus influenzae, Neisseria gonorrhoeee, Moraxella catarrahlis, Salmonella sps., Chlamydia trachomatis, Peptostreptococcus productus, Peptostreptococcus anaerobius, Lactobacillusfermentum, Eubacterium lentum, Candida glabrata, Candida albicans, Chlamydia spp., Camplobacter spp., Salmonella spp., smallpox (variola major), Yersina Pestis, Herpes Simplex Virus I (HSV I), and Herpes Simplex Virus II (HSV II).

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of Group B Streptococcus.

In various embodiments, a method of carrying out PCR on a sample can further include one or more of the following steps: heating the biological sample in the microfluidic cartridge; pressurizing the biological sample in the microfluidic cartridge at a pressure differential compared to ambient pres-

sure of between about 20 kilopascals and 200 kilopascals, or in some embodiments, between about 70 kilopascals and 110 kilopascals

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In some embodiments, the method for sampling a polynucleotide can include the steps of: placing a microfluidic 5 cartridge containing a PCR-ready sample in a receiving bay of a suitably configured apparatus; carrying out PCR on the sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide in the sample, the PCR-ready sample comprising a polymerase 10 enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid, and a plurality of nucleotides; contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the at least one fluorogenic probe that is selective for a polynucleotide 15 sequence, wherein the probe is selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses; and detecting the fluorogenic probe, the presence of the organism for which the 20 one fluorogenic probe is selective is determined.

Carrying out PCR on a PCR-ready sample can additionally include: independently contacting each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; and/or contacting the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon 30 thereof with at least one probe that is selective for a polynucleotide sequence.

In various embodiments, a method of using the apparatus and cartridge described herein can further include one or more of the following steps: determining the presence of a 35 polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; determining that the sample was contaminated if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof; and/or in some embodiments, wherein the PCR reagent mixture further comprises a positive control plasmid and a plasmid probe selective for at least a portion of the plasmid, the method further including determining that a PCR amplification has occurred 45 if the plasmid probe is detected.

In various embodiments, the microfluidic cartridge as described herein can be provided in the form of a kit, wherein the kit can include a microfluidic cartridge, and a liquid 50 transfer member (such as a syringe or a pipette). In various embodiments, the kit can further include instructions to employ the liquid transfer member to transfer a sample containing extracted nucleic acid from a sample container via a sample inlet to the microfluidic network on the microfluidic 55 cartridge. In some embodiments, the microfluidic cartridge and the liquid transfer member can be sealed in a pouch with an inert gas.

Typically when transferring a sample from liquid dispenser, such as a pipette tip, to an inlet on the microfluidic 60 cartridge, a volume of air is simultaneously introduced into the microfluidic network, the volume of air being between about 0.5 mL and about 5 mL. Presence of air in the microfluidic network, however, is not essential to operation of the cartridge described herein.

In various embodiments, the kit can further include at least one computer-readable label on the cartridge. The label can 30

include, for example, a bar code, a radio frequency tag or one or more computer-readable characters. When used in conjunction with a similar computer-readable label on a sample container, such as a vial or a pouch, matching of diagnostic results with sample is thereby facilitated.

In some embodiments, a sample identifier of the apparatus described elsewhere herein is employed to read a label on the microfluidic cartridge and/or a label on the biological sample. Heater Unit

An exemplary heater unit 2020 is shown in FIG. 26. The unit is configured to deliver localized heat to various selected regions of a cartridge received in a receiving bay 2014. Heater unit 2020 is configured to be disposed within a diagnostic apparatus during operation, as further described herein, and in certain embodiments is removable from that apparatus, for example to facilitate cleaning, or to permit reconfiguration of the heater circuitry. In various embodiments, heater unit 2020 can be specific to particular designs of microfluidic networks and microfluidic substrate layouts.

Shown in FIG. 26 is a heater unit having a recessed surface 2044 that provides a platform for supporting a microfluidic cartridge when in receiving bay 2014. In one embodiment, the cartridge rests directly on surface 2044. Surface 2044 is shown as recessed, in FIG. 2, but need not be so and, for example, may be raised or may be flush with the surrounding area of the heater unit. Surface 2044 is typically a layer of material that overlies a heater chip or board, or a heater substrate, that contains heater micro-circuitry configured to selectively and specifically heat regions of a microfluidic substrate, such as in a cartridge, in the receiving bay 2014.

Area 2044 is configured to accept a microfluidic cartridge in a single orientation. Therefore area 2044 can be equipped with a registration member such as a mechanical key that prevents a user from placing a cartridge into receiving bay 2014 in the wrong configuration. Shown in FIG. 26 as an exemplary mechanical key 2045 is a diagonally cutout corner of area 2044 into which a complementarily cutoff corner of a microfluidic cartridge fits. Other registration members are consistent with the heater unit described herein, for example, a feature engineered on one or more edges of a cartridge including but not limited to: several, such as two or more, cut-out corners, one or more notches cut into one or more edges of the cartridge; or one or more protrusions fabricated into one or more edges of the cartridge. Alternative registration members include one or more lugs or bumps engineered into an underside of a cartridge, complementary to one or more recessed sockets or holes in surface 2044 (not shown in the embodiment of FIG. 26). Alternative registration members include one or more recessed sockets or holes engineered into an underside of a cartridge, complementary to one or more lugs or bumps on surface 2044. In general, the pattern of features is such that the cartridge possesses at least one element of asymmetry so that it can only be inserted in a single orientation into the receiving bay.

Also shown in FIG. 26 is a hand-grasp 2042 that facilitates removal and insertion of the heater unit into an apparatus by a user. Cutaway 2048 permits a user to easily remove a cartridge from receiving bay 2014 after a processing run where, e.g., a user's thumb or finger when grabbing the top of the cartridge, is afforded comfort space by cutaway 2048. Both cutaways 2042 and 2048 are shown as semicircular recesses in the embodiment of FIG. 26, but it would be understood that they are not so limited in shape. Thus, rectangular, square, triangular, half-oval, contoured, and other shaped recesses are also consistent with a heater unit as described herein.

In the embodiment of FIG. 26, which is designed to be compatible with an exemplary apparatus as further described

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herein, the front of the heater unit is at the left of the figure. At the rear of heater unit 2020 is an electrical connection 2050, such as an RS-232 connection, that permits electrical signals to be directed to heaters located at specific regions of area 2044 during sample processing and analysis, as further described herein. Thus, underneath area 2044 and not shown in FIG. 2 can be an array of heat sources, such as resistive heaters, that are configured to align with specified locations of a microfluidic cartridge properly inserted into the receiving bay. Surface 2044 is able to be cleaned periodically, for example with common cleaning agents (e.g., a 10% bleach solution), to ensure that any liquid spills that may occur during sample handling do not cause any short circuiting. Such cleaning can be carried out frequently when the heater 15 unit is disposed in a diagnostic apparatus, and less frequently but more thoroughly when the unit is removed.

Other non-essential features of heater unit 2020 are as follows. One or more air vents 2052 can be situated on one or more sides (such as front, rear, or flanking) or faces (such as 20 top or bottom) of heater unit 2020, to permit excess heat to escape, when heaters underneath receiving bay 2014, are in operation. The configuration of air vents in FIG. 26, as a linear array of square vents, is exemplary and it would be understood that other numbers and shapes thereof are consistent 25 with routine fabrication and use of a heater unit. For example, although 5 square air vents are shown, other numbers such as 1, 2, 3, 4, 6, 8, or 10 air vents are possible, arranged on one side, or spread over two or more sides and/or faces of the heater unit. In further embodiments, air vents may be circular, rectangular, oval, triangular, polygonal, and having curved or squared vertices, or still other shapes, including irregular shapes. In further embodiments two or more vents need not be disposed in a line, parallel with one another and with an edge of the heater unit but may be disposed offset from one another.

Heater unit 2020 may further comprise one or more guiding members 2047 that facilitate inserting the heater unit into an apparatus as further described herein, for an embodiment in which heater unit 2020 is removable by a user. Heater unit 40 is advantageously removable because it permits system 2000 to be easily reconfigured for a different type of analysis, such as employing a different cartridge with a different registration member and/or microfluidic network, in conjunction with the same or a different sequence of processing operations. In 45 other embodiments, heater unit 2020 is designed to be fixed and only removable, e.g., for cleaning, replacement, or maintenance, by the manufacturer or an authorized maintenance agent, and not routinely by the user. Guiding members 2047 may perform one or more roles of ensuring that the heater unit 50 is aligned correctly in the apparatus, and ensuring that the heater unit makes a tight fit and does not significantly move during processing and analysis of a sample, or during transport of the apparatus.

Guiding members shown in the embodiment of FIG. **26** are 55 on either side of receiving bay **2044** and stretch along a substantial fraction of the length of unit **2020**, but such an arrangement of guiding members is exemplary. Other guiding members are consistent with use herein, and include but are not limited to other numbers of guiding members such as 1, 3, 60 4, 5, 6, or 8, and other positions thereof, including positioned in area **2051** of unit **2020**, and need not stretch along as much of the length of unit **2020** as shown in FIG. **26**, or may stretch along its entire length. Guiding members **2047** are shown having a non-constant thickness along their lengths. It is 65 consistent herein that other guiding members may have essentially constant thickness along their lengths. At the end

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of the heater unit that is inserted into an apparatus, in the embodiment shown, the edges are beveled to facilitate proper placement.

Also shown in FIG. 26 is an optional region of fluorescent material, such as optically fluorescent material 2069, on area 2051 of heater unit 2020. The region of fluorescent material is configured to be detected by a detection system further described herein. The region 2069 is used for verifying the state of optics in the detection system prior to sample processing and analysis and therefore acts as a control, or a standard. For example, in one embodiment a lid of the apparatus in which the heater unit is disposed, when in an open position, permits ambient light to reach region 2069 and thereby cause the fluorescent material to emit a characteristic frequency or spectrum of light that can be measured by the detector for, e.g., standardization or calibration purposes. In another embodiment, instead of relying on ambient light to cause the fluorescent material to fluoresce, light source from the detection system itself, such as one or more LED's, is used to shine on region 2069. The region 2069 is therefore positioned to align with a position of a detector. Region 2069 is shown as rectangular, but may be configured in other shapes such as square, circular, elliptical, triangular, polygonal, and having curved or squared vertices. It is also to be understood that the region 2069 may be situated at other places on the heater unit 2020, according to convenience and in order to be complementary to the detection system deployed.

In particular and not shown in FIG. 26, heater/sensor unit 2020 can include, for example, a multiplexing function in a discrete multiplexing circuit board (MUX board), one or more heaters (e.g., a microheater), one or more temperature sensors (optionally combined together as a single heater/ sensor unit with one or more respective microheaters, e.g., as photolithographically fabricated on fused silica substrates). The micro-heaters can provide thermal energy that can actuate various microfluidic components on a suitably positioned microfluidic cartridge. A sensor (e.g., as a resistive temperature detector (RTD)) can enable real time monitoring of the micro-heaters, for example through a feedback based mechanism to allow for rapid and accurate control of the temperature. One or more microheaters can be aligned with corresponding microfluidic components (e.g., valves, pumps, gates, reaction chambers) to be heated on a suitably positioned microfluidic cartridge. A microheater can be designed to be slightly bigger than the corresponding microfluidic component(s) on the microfluidic cartridge so that even though the cartridge may be slightly misaligned, such as off-centered, from the heater, the individual components can be heated effectively.

Heater Configurations to Ensure Uniform Heating of a Region

The microfluidic substrates described herein are configured to accept heat from a contact heat source, such as found in a heater unit described herein. The heater unit typically comprises a heater board or heater chip that is configured to deliver heat to specific regions of the microfluidic substrate, including but not limited to one or more microfluidic components, at specific times. For example, the heat source is configured so that particular heating elements are situated adjacent to specific components of the microfluidic network on the substrate. In certain embodiments, the apparatus uniformly controls the heating of a region of a microfluidic network. In an exemplary embodiment, multiple heaters can be configured to simultaneously and uniformly heat a region, such as the PCR reaction chamber, of the microfluidic sub-

strate. The term heater unit, as used herein, may be used interchangeably to describe either the heater board or an item

such as shown in FIG. 26. Referring to FIGS. 27A and 27B, an exemplary set of

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heaters configured to heat, cyclically, PCR reaction chamber 5 1001 is shown. It is to be understood that heater configurations to actuate other regions of a microfluidic cartridge such as other gates, valves, and actuators, may be designed and deployed according to similar principles to those governing the heaters shown in FIGS. 27A and 27B.

Referring to FIGS. 27A and 27B, an exemplary PCR reaction chamber 1001 in a microfluidic substrate, typically a chamber or channel having a volume ~1.6 μl, is configured with a long side and a short side, each with an associated heating element. A PCR reaction chamber may also be 15 referred to as a PCR reactor, herein, and the region of a cartridge in which the reaction chamber is situated may be called a zone. The heater substrate therefore includes four heaters disposed along the sides of, and configured to heat, a given PCR reaction chamber, as shown in the exemplary 20 embodiment of FIG. 27A: long top heater 1005, long bottom heater 1003, short left heater 1007, and short right heater 1009. The small gap between long top heater 1005 and long bottom heater 1003 results in a negligible temperature gradient (less than 1° C. difference across the width of the PCR 25 channel at any point along the length of the PCR reaction chamber) and therefore an effectively uniform temperature throughout the PCR reaction chamber. The heaters on the short edges of the PCR reactor provide heat to counteract the gradient created by the two long heaters from the center of the 30 reactor to the edge of the reactor.

It would be understood by one of ordinary skill in the art that still other configurations of one or more heater(s) situated about a PCR reaction chamber are consistent with the methods and apparatus described herein. For example, a 'long' 35 for a single PCR reaction chamber, can also be applied to a side of the reaction chamber can be configured to be heated by two or more heaters. Specific orientations and configurations of heaters are used to create uniform zones of heating even on substrates having poor thermal conductivity because the poor thermal conductivity of glass, or quartz, polyimide, FR4, 40 ceramic, or fused silica substrates is utilized to help in the independent operation of various microfluidic components such as valves and independent operation of the various PCR lanes. It would be further understood by one of ordinary skill in the art, that the principles underlying the configuration of 45 heaters around a PCR reaction chamber are similarly applicable to the arrangement of heaters adjacent to other components of the microfluidic cartridge, such as actuators, valves,

Generally, the heating of microfluidic components, such as 50 a PCR reaction chamber, is controlled by passing currents through suitably configured microfabricated heaters. Under control of suitable circuitry, the lanes of a multi-lane cartridge can then be controlled independently of one another. This can lead to a greater energy efficiency of the apparatus, because 55 not all heaters are heating at the same time, and a given heater is receiving current for only that fraction of the time when it is required to heat. Control systems and methods of controllably heating various heating elements are further described in U.S. patent application Ser. No. 11/940,315, filed Nov. 14, 60 2007 and entitled "Heater Unit for Microfluidic Diagnostic System".

In certain embodiments, each heater has an associated temperature sensor. In the embodiment of FIG. 27A, a single temperature sensor 1011 is used for both long heaters. A 65 temperature sensor 1013 for short left heater, and a temperature sensor 1015 for short right heater are also shown. The

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temperature sensor in the middle of the reactor is used to provide feedback and control the amount of power supplied to the two long heaters, whereas each of the short heaters has a dedicated temperature sensor placed adjacent to it in order to control it. As further described herein, temperature sensors are preferably configured to transmit information about temperature in their vicinity to a processor in the apparatus at such times as the heaters are not receiving current that causes them to heat. This can be achieved with appropriate control of 10 current cycles.

In order to reduce the number of sensor or heater elements required to control a PCR heater, the heaters may be used to sense as well as heat, and thereby obviate the need to have a separate dedicated sensor for each heater. In another embodiment, each of the four heaters may be designed to have an appropriate wattage, and connect the four heaters in series or in parallel to reduce the number of electronically-controllable elements from four to just one, thereby reducing the burden on the associated electronic circuitry.

FIG. 27B shows expanded views of heaters and temperature sensors used in conjunction with a PCR reaction chamber of FIG. 27A. Temperature sensors 1001 and 1013 are designed to have a room temperature resistance of approximately 200-300 ohms. This value of resistance is determined by controlling the thickness of the metal layer deposited (e.g., a sandwich of 400 Å TiW/3,000 Å Au/400 Å TiW), and etching the winding metal line to have a width of approximately 10-25 µm and 20-40 mm length. The use of metal in this layer gives it a temperature coefficient of resistivity of the order of 0.5-20° C./ohms, preferably in the range of 1.5-3° C./ohms. Measuring the resistance at higher temperatures enables determination of the exact temperature of the location of these sensors.

The configuration for uniform heating, shown in FIG. 27A multi-lane PCR cartridge in which multiple independent PCR reactions occur.

Each heater can be independently controlled by a processor and/or control circuitry used in conjunction with the apparatus described herein. FIG. 27C shows thermal images, from the top surface of a microfluidic cartridge when heated by heaters configured as in FIGS. 27A and 27B, when each heater in turn is activated, as follows: (A): Long Top only; (B) Long Bottom only; (C). Short Left only; (D) Short Right only; and (E) All Four Heaters on. Panel (F) shows a view of the reaction chamber and heaters on the same scale as the other image panels in FIG. 27C. Also shown in the figure is a temperature bar.

The configuration for uniform heating, shown in FIG. 27A for a single PCR reaction chamber, can be applied to a multilane PCR cartridge in which multiple independent PCR reactions occur. See, e.g., FIG. 28, which shows an array of heater elements suitable to heat a cartridge herein.

Heater Multiplexing (Under Software Control)

Another aspect of the heater unit described herein, relates to a control of heat within the system and its components. The method leads to a greater energy efficiency of the apparatus described herein, because not all heaters are heating at the same time, and a given heater is receiving current for only part of the time.

Generally, the heating of microfluidic components, such as a PCR reaction chamber, is controlled by passing currents through suitably configured microfabricated heaters. The heating can be further controlled by periodically turning the current on and off with varying pulse width modulation (PWM), wherein pulse width modulation refers to the ontime/off-time ratio for the current. The current can be sup-

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35 plied by connecting a microfabricated heater to a high voltage

source (for example, 30 V), which can be gated by the PWM signal. In some embodiments, the device includes 48 PWM signal generators. Operation of a PWM generator includes generating a signal with a chosen, programmable, period (the 5 end count) and a particular granularity. For instance, the signal can be 4000 µs (micro-seconds) with a granularity of 1 μs, in which case the PWM generator can maintain a counter beginning at zero and advancing in increments of 1 µs until it reaches 4000 µs, when it returns to zero. Thus, the amount of heat produced can be adjusted by adjusting the end count. A high end count corresponds to a greater length of time during which the microfabricated heater receives current and therefore a greater amount of heat produced. It would be understood that the granularity and signal width can take values 15 other than those provided here without departing from the principles described herein.

Fluorescence Detection System, Including Lenses and Filters, and Multiple Parallel Detection for a Multi-Lane Cartridge

The detection system herein is configured to monitor fluorescence coming from one or more species involved in a biochemical reaction. The system can be, for example, an optical detector having a light source that selectively emits detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof, as further described elsewhere herein. Alternatively, the optical detector can include a bandpass-filtered diode that selectively 30 emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent 35 emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations of, for example, a microfluidic sub- 40 strate, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. The detector further has potential for 2, 3 or 4 color detection and is controlled by software, preferably custom software, configured to sample information from the detector.

The detection system described herein is capable of detecting a fluorescence signal from nanoliter scale PCR reactions. Advantageously, the detector is formed from inexpensive components, having no moving parts. The detector can be configured to couple to a microfluidic cartridge as further 50 described herein, and can also be part of a pressure application system, such as a sliding lid on an apparatus in which the detector is situated, that keeps the cartridge in place.

FIGS. **29-31**B depict an embodiment of a highly sensitive fluorescence detection system that includes light emitting 55 diodes (LED's), photodiodes, and filters/lenses for monitoring, in real-time, one or more fluorescent signals emanating from the microfluidic channel. The embodiment in FIGS. 29-31B displays a two-color detection system having a modular design that couples with a single microfluidic channel 60 found, for example, in a microfluidic cartridge. It would be understood by one skilled in the art that the description herein could also be adapted to create a detector that just detects a single color of light. FIGS. 31A and 31B show elements of optical detector elements 1220 including light sources 1232 65 (for example, light emitting diodes), lenses 1234, light detectors 1236 (for example, photodiodes) and filters 1238. The

detector comprises two LED's (blue and red, respectively) and two photodiodes. The two LED's are configured to transmit a beam of focused light on to a particular region of the cartridge. The two photodiodes are configured to receive light that is emitted from the region of the cartridge. One photodiode is configured to detect emitted red light, and the other photodiode is configured to detect emitted blue light. Thus, in this embodiment, two colors can be detected simultaneously from a single location. Such a detection system can be configured to receive light from multiple microfluidic channels

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by being mounted on an assembly that permits it to slide over and across the multiple channels. The filters can be, for example, bandpass filters, the filters at the light sources corresponding to the absorption band of one or more fluorogenic probes and the filters at the detectors corresponding to the emission band of the fluorogenic probes.

FIGS. 32 and 33 show an exemplary read-head comprising a multiplexed 2 color detection system that is configured to mate with a multi-lane microfluidic cartridge. FIG. 32 shows 20 a view of the exterior of a multiplexed read-head. FIG. 33 is an exploded view that shows how various detectors are configured within an exemplary multiplexed read head, and in communication with an electronic circuit board.

Each of the detection systems multiplexed in the assembly light in an absorption band of a fluorescent dye, and a light 25 of FIGS. 32 and 33 is similar in construction to the embodiment of FIGS. 29-31B. The module in FIGS. 32 and 33 is configured to detect fluorescence from each of 12 microfluidic channels, as found in, for example, the respective lanes of a 12-lane microfluidic cartridge. Such a module therefore comprises 24 independently controllable detectors, arranged as 12 pairs of identical detection elements. Each pair of elements is then capable of dual-color detection of a pre-determined set of fluorescent probes. It would be understood by one of ordinary skill in the art that other numbers of pairs of detectors are consistent with the apparatus described herein. For example, 4, 6, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 pairs are also consistent and can be configured according to methods and criteria understood by one of ordinary skill in the

Detection Sensitivity, Time Constant and Gain

A typical circuit that includes a detector as described herein includes, in series, a preamplifier, a buffer/inverter, a filter, and a digitizer. Sensitivity is important so that high gain is very desirable. In one embodiment of the preamplifier, a very large, for example 100 G $\Omega$ , resistor is placed in parallel with the diode. Other values of a resistor would be consistent with the technology herein: such values typically fall in the range  $0.5\text{-}100\,\mathrm{G}\Omega$ , such as  $1\text{-}50\,\mathrm{G}\Omega$ , or  $2\text{-}10\,\mathrm{G}\Omega$ ). An exemplary pre-amplifier circuit configured in this way is shown in FIG. 7. Symbols in the figure have their standard meanings in electronic circuit diagrams.

The FIG. 34 shows a current-to-voltage converter/pre-amplifier circuit suitable for use with the detection system. D11 is the photodetector that collects the fluorescent light coming from the microfluidic channel and converts it into an electric current. The accompanying circuitry is used to convert these fluorescent currents into voltages suitable for measurement and output as a final measure of the fluorescence.

A resistor-capacitor circuit in FIG. 34 contains capacitor C45 and resistor R25. Together, the values of capacitance of C45 and resistance of R25 are chosen so as to impact the time constant  $\tau_c$  (equal to the product of R25 and C45) of the circuit as well as gain of the detection circuit. The higher the time constant, the more sluggish is the response of the system to incident light. It typically takes the duration of a few time constants for the photodetector to completely charge to its maximum current or to discharge to zero from its saturation

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value. It is important for the photo current to decay to zero between measurements, however. As the PCR systems described herein are intended to afford rapid detection measurements, the product  $R_{25}C_{45}$  should therefore be made as low as possible. However, the gain of the pre-amplifier whose circuitry is shown is directly proportional to the (fluorescentactivated) current generated in the photodetector and the resistance R<sub>25</sub>. As the fluorescence signal from the microfluidic channel device is very faint (due to low liquid volume as well as small path lengths of excitation), it is thus important to maximize the value of R25. In some embodiments, R25 is as high as 100 Giga-Ohms (for example, in the range 10-100  $G\Omega$ ), effectively behaving as an open-circuit. With such values, the time-constant can take on a value of approximately  $_{15}$ 50-100 ms by using a low-value capacitor for C45. For example, the lowest possible available standard off-the-shelf capacitor has a value of 1 pF (1 picoFarad). A time-constant in the range 50-100 ms ensures that the photocurrent decays to zero in approximately 0.5 s (approx. 6 cycles) and therefore 20 that approximately 2 samplings can be made per second. Other time constants are consistent with effective use of the technology herein, such as in the range 10 ms-1 s, or in the range 50 ms-500 ms, or in the range 100-200 ms. The actual time constant suitable for a given application will vary 25 according to circumstance and choice of capacitor and resistor values. Additionally, the gain achieved by the pre-amplifier circuit herein may be in the range of  $10^7$ – $5\times10^9$ , for example may be  $1\times10^9$ .

As the resistance value for R25 is very high ( $\sim$ 100 G $\Omega$ ), the manner of assembly of this resistor on the optics board is important for the overall efficiency of the circuit. Effective cleaning of the circuit during assembly and before use is important to achieve an optimal time-constant and gain for 35 the optics circuit.

It is also important to test each photo-diode that is used, because many do not perform according to specification. Sensitivity and Aperturing

through the sample in the micro-fluidic channel (as described herein, typically 300µ deep). This is a very small optical path-length for the light in the sample. The generated fluorescence then also goes through a second filter, and into a photo-detector. Ultimately, then, the detector must be capable 45 of detecting very little fluorescence. Various aspects of the detector configuration can improve sensitivity, however.

The illumination optics can be designed so that the excitation light falling on the PCR reactor is incident along an area that is similar to the shape of the reactor. As the reactor is 50 typically long and narrow, the illumination spot should be long and narrow, i.e., extended, as well. The length of the spot can be adjusted by altering a number of factors, including: the diameter of the bore where the LED is placed (the tube that holds the filter and lens has an aperturing effect); the distance 55 of the LED from the PCR reactor; and the use of proper lens at the right distance in between. As the width of the beam incident on the reactor is determined by the bore of the optical element (approximately 6 mm in diameter), it is typical to use an aperture (a slit having a width approximately equal to the 60 width of the reactor, and a length equal to the length of the detection volume) to make an optimal illumination spot. A typical spot, then, is commensurate with the dimensions of a PCR reaction chamber, for example 1.5 mm wide by 7 mm long. FIG. 35A shows the illumination spot across 12 PCR reactors for the two different colors used. A typical aperture is made of anodized aluminum and has very low autofluores38

cence in the wavelengths of interest. FIG. 35B illustrates a cross-section of a detector, showing an exemplary location for an aperture 802.

The optimal spot size and intensity is importantly dependent on the ability to maintain the correct position of the LED's with respect to the center of the optical axis. Special alignment procedures and checks can be utilized to optimize this. The different illuminations can also be normalized with respect to each other by adjusting the power current through each of the LED's or adjusting the fluorescence collection time (the duration for which the photodetector is on before measuring the voltage) for each detection spot. It is also important to align the detectors with the axis of the microchannels.

The aperturing is also important for successful fluorescence detection because as the cross-sectional area of the incident beam increases in size, so the background fluorescence increases, and the fluorescence attributable only to the molecules of interest (PCR probes) gets masked. Thus, as the beam area increases, the use of an aperture increases the proportion of collected fluorescence that originates only from the PCR reactor. Note that the aperture used in the detector herein not only helps collect fluorescence only from the reaction volume but it correspondingly adjusts the excitation light to mostly excite the reaction volume. The excitation and emission aperture is, of course, the same.

Based on a typical geometry of the optical exctiation and emission system and aperturing, show spot sizes as small as 0.5 mm by 0.5 mm and as long as 8 mm×1.5 mm have been found to be effective. By using a long detector (having an active area 6 mm by 1 mm) and proper lensing, the aperture design can extend the detection spot to as long as 15-20 mm, while maintaining a width of 1-2 mm using an aperture. Correspondingly, the background fluorescence decreases as the spot size is decreased, thereby increasing the detection sensitivity.

Use of Detection System to Measure/Detect Fluid in PCR Chamber

The fluorescence detector is sensitive enough to be able to The LED light passes through a filter before passing 40 collect fluorescence light from a PCR chamber of a microfluidic substrate. The detector can also be used to detect the presence of liquid in the chamber, a measurement that provides a determination of whether or not to carry out a PCR cycle for that chamber. For example, in a multi-sample cartridge, not all chambers will have been loaded with sample; for those that are not, it would be unnecessary to apply a heating protocol thereto. One way to determine presence or absence of a liquid is as follows. A background reading is taken prior to filling the chamber with liquid. Another reading is taken after microfluidic operations have been performed that should result in filling the PCR chamber with liquid. The presence of liquid alters the fluorescence reading from the chamber. A programmable threshold value can be used to tune an algorithm programmed into a processor that controls operation of the apparatus as further described herein (for example, the second reading has to exceed the first reading by 20%). If the two readings do not differ beyond the programmed margin, the liquid is deemed to not have entered the chamber, and a PCR cycle is not initiated for that chamber. Instead, a warning is issued to a user.

**Exemplary Electronics and Software** 

The heater unit described herein can be controlled by various electronics circuitry, itself operating on receipt of computer-controlled instructions. FIG. 36 illustrates exemplary electronics architecture modules for operating a heater unit and diagnostic apparatus. It would be understood by one of ordinary skill in the art that other configurations of electronics 39

components are consistent with operation of the apparatus as described herein. In the exemplary embodiment, the electronics architecture is distributed across two components of the apparatus: the Analyzer 2100 and a Heater unit 2102. The Analyzer apparatus as further described herein contains, for 5 example, an Optical Detection Unit 2108, a Control Board 2114, a Backplane 2112, and a LCD Touchscreen 2110. The Control Board includes a Card Engine 2116 further described herein, and Compact Flash memory 2118, as well as other components. The Heater Assembly includes a Heater Board 2104 and a Heater Mux Board 2106, both further described

In one embodiment, the Card Engine electronics module 2116 is a commercial, off the shelf "single board computer"  $_{15}$ containing a processor, memory, and flash memory for operating system storage.

The optional LCD+Touchscreen electronics module 2110 is a user interface, for example, driven through a touchscreen, such as a 640 pixel by 480 pixel 8 inch LCD and 5-wire 20

The Compact Flash electronics module 2118 is, for example, a 256 megabyte commercial, off the shelf, compact flash module for application and data storage. Other media are alternatively usable, such as USB-drive, smart media 25 circuitry that can be employed to collect, digitize, filter, and card, memory stick, and smart data-card having the same or other storage capacities.

The Backplane electronics module 2112 is a point of connection for the removable heater assembly 2102. Bare PC boards with two connectors are sufficient to provide the nec- 30 essary level of connectivity.

The Control Board electronics module 2114 supports peripherals to the Card Engine electronics module 2116. In one embodiment, the peripherals include such devices as a USB host+slave or hub, a USB CDROM interface, serial 35 ports, and ethernet ports. The Control Board 2114 can include a power monitor with a dedicated processor. The Control Board may also include a real time clock. The Control Board may further include a speaker. The Control Board 2114 also includes a CPLD to provide SPI access to all other modules 40 and programming access to all other modules. The Control Board includes a programmable high voltage power supply. The Control Board includes a Serial-Deserializer interface to the LCD+Touchscreen electronics module 2110 and to the Optical Detection Unit electronics module 2108. The Control 45 Board also includes module connectors.

In the exemplary embodiment, the optical detection unit electronics module 2108 contains a dedicated processor. The optical detection unit 2108 contains a serializer-deserializer interface. The optical detection unit 2108 contains LED driv- 50 ers. The optical detection unit also contains high gain-low noise photodiode amplifiers. The optical detection unit can have power monitoring capability. The optical detection unit can also be remotely reprogrammable.

The Heater Board electronics module 2104 is preferably a 55 glass heater board. The Heater Board has PCB with bonding pads for glass heater board and high density connectors.

In one embodiment, the heater mux ('multiplex') board electronics module 2106 has 24 high-speed ADC, 24 precision current sources, and 96 optically isolated current drivers 60 for heating. The heater mux board has the ability to timemultiplex heating/measurement. The heater mux board has multiplexer banks to multiplex inputs to ADC, and to multiplex current source outputs. The heater mux board has a FPGA with a soft processor core and SDRAM. The heater 65 mux board has a Power Monitor with a dedicated processor. The Heater Mux Board can be remotely reprogrammable.

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In another embodiment, control electronics can be spread over four different circuit board assemblies. These include the MAIN board: Can serve as the hub of the Analyzer control electronics and manages communication and control of the other various electronic sub-assemblies. The main board can also serve as the electrical and communications interface with the external world. An external power supply (12V DC/10 A: UL certified) can be used to power the system. The unit can communicate via 5 USB ports, a serial port and an Ethernet port. Finally, the main board can incorporate several diagnostic/safety features to ensure safe and robust operation of the

MUX Board: Upon instruction from the main board, the MUX board can perform all the functions typically used for accurate temperature control of the heaters and can coordinate the collection of fluorescence data from the detector board.

LCD Board: Can contain the typical control elements to light up the LCD panel and interpret the signals from the touch sensitive screen. The LCD/touch screen combination can serve as a mode of interaction with the user via a Graphical User Interface.

Detector Board: Can house typical control and processing transmit the data from the fluorescence detection modules.

Certain software can be executed in each electronics module. The Control Board Electronics Module executes, for example, Control Board Power Monitor software. The Card Engine electronics module executes an operating system, graphical user interface (GUI) software, an analyzer module, and an application program interface (api). The Optical Detection Unit electronics module executes an optics software module. The Heater Mux Board electronics module executes dedicated Heater Mux software, and Heater Mux Power Monitor software. Each of the separate instances of software can be modular and under a unified control of, for example, driver software.

The exemplary electronics can use Linux, UNIX, Windows, or MacOS, including any version thereof, as the operating system. The operating system is preferably loaded with drivers for USB, Ethernet, LCD, touchscreen, and removable media devices such as compact flash. Miscellaneous programs for configuring the Ethernet interface, managing USB connections, and updating via CD-ROM can also be included.

In the embodiment of FIG. 36, the analyzer module is the driver for specific hardware. The analyzer module provides access to the Heater Mux Module, the Optical Detection Unit, the Control Board Power Monitor, the Real Time Clock, the High Voltage Power Supply, and the LCD backlight. The analyzer module provides firmware programming access to the Control Board power monitor, the Optical Detection Unit, and the Heater Mux Module.

The API provides uniform access to the analyzer module driver. The API is responsible for error trapping, and interrupt handling. The API is typically programmed to be thread safe.

The GUI software can be based on a commercial, off-theshelf PEG graphics library. The GUI can use the API to coordinate the self-test of optical detection unit and heater assembly. The GUI starts, stops, and monitors test progress. The GUI can also implement an algorithm to arrive on diagnosis from fluorescence data. The GUI provides access control to unit and in some embodiments has an HIS/LIS interface.

The Control Board Power Monitor software monitors power supplies, current and voltage, and signals error in case of a fault.

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The Optics Software performs fluorescence detection which is precisely timed to turn on/off of LED with synchronous digitization of the photodetector outputs. The Optics Software can also monitor power supply voltages. The Optics Software can also have self test ability.

The Heater Mux Module software implements a "protocol player" which executes series of defined "steps" where each "step" can turn on sets of heaters to implement a desired microfluidic action. The Heater Mux Module software also has self test ability. The Heater Mux Module software contains a fuzzy logic temperature control algorithm.

The Heater Mux Power Monitor software monitors voltage and current levels. The Heater Mux Power Monitor software can participate in self-test, synchronous, monitoring of the current levels while turning on different heaters.

#### **EXAMPLES**

The following are exemplary aspects of various parts and  $_{20}$  functions of the system described herein.

Additional embodiments of a cartridge are found in U.S. patent application Ser. No. 11/940,310, entitled "Microfluidic Cartridge and Method of Making Same", and filed on even date herewith, the specification of which is incorporated 25 herein by reference.

Additional embodiments of heater units and arrays are described in U.S. patent application Ser. No. 11/940,315, entitled "Heater Unit for Microfluidic Diagnostic System" and filed on even date herewith, the specification of which is incorporated herein by reference in its entirety.

Further description of suitably configured detectors are described in U.S. patent application Ser. No. 11/940,321, filed on Nov. 14, 2007 and entitled "Fluorescence Detector for Microfluidic Diagnostic System", incorporated herein by reference.

#### Example 1

#### Analyzer Having Removable Heater Unit

This non-limiting example describes pictorially, various embodiments of an apparatus, showing incorporation of a heater unit and a microfluidic cartridge operated on by the heater unit

FIG. 37 shows an apparatus 1100 that includes a housing having a display output 1102, an openable lid 1104, and a bar code reader 1106. The cartridge is positioned in a single orientation in a receiving bay under the lid, FIG. 38. The lid of the apparatus can be closed to apply pressure to the cartridge, as shown in FIG. 39. The unit currently weighs about 20 lbs. and is approximately 10" wide by 16" deep by 13" high.

FIGS. **40** and **41**: The heating stage of the apparatus can be 55 removable for cleaning, maintenance, or to replace a custom heating stage for a particular microfluidic cartridge. FIGS. **40** and **41** also show how a heater unit is insertable and removable from a front access door to an analyzer apparatus.

#### Example 2

#### Assembly of an exemplary Heater Unit

FIG. **42**A shows an exploded view of an exemplary heater unit. The unit has a top cover and a bottom cover that together

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enclose a Mux board (control board), a pressure support layer, and insulator film, and a microthermal circuit on a PCB. The last of these is the heat source that selectively heats regions of a microfluidic substrate placed in contact therewith through the top cover.

An exemplary heater substrate, FIG. 42B, consists of a photo-lithographically processed glass wafer bonded to a standard 0.100" standard FR4 printed circuit board. The glass wafer is 0.5 mm thick and is cut into a rectangle the size of ~3.5×4.25 inches. The glass substrate has numerous metal heaters and resistive temperature sensors photo-lithographically etched on the surface of the glass wafer. The substrate is aligned and bonded to the PCBoard using a compliant epoxy, ensuring flatness to within 2-3 mils over the surface of the wafer. The cured epoxy should withstand up to 120° C. for two hours minimum. Approximately 300-400 bond pads of the size of approximately 1 mm×0.25 mm, with exposed gold surfaces, are located along the two long edges of the wafer. These pads are wirebonded (ball-bonding) to corresponding pads on the PCB using 1.5 mil gold wires. Wire bonding is a threading process, standard in semiconductor FAB. Alternatively, a flip-chip method may be used, though such methods are more complicated and may warp the wafer because of thermal mismatch. Wire bonds should have good integrity and pass defined pull strength. The substrate is baked at 120° C. for two hours and then the wire bonds are encapsulated by a compliant epoxy that will protect the wirebonds but not damage the bonds even at 120° C. Encapsulant should not spill over pre-defined area around the wirebonds and should not be taller than a defined height. For example, instead of laying epoxy all over the substrate, lines (e.g., a hash pattern) of it are made so that epoxy cures and air escapes through side. Alternatively, a laminate fill (adhesive on both sides) can be used. Standard connectors are soldered to the PCB and then the unit is tested using a test set-up to ensure all heaters and sensors read the right resistance values.

Pictures of an exemplary Mux board and assembled heater unit are shown in FIGS. **27-29**.

#### Example 3

#### Pulse Width Modulation for Heater Circuitry

In various embodiments, the operation of a PWM generator can also include a programmable start count in addition to the aforementioned end count and granularity. In such embodiments, multiple PWM generators can produce signals that can be selectively non-overlapping (e.g., by multiplexing the on-time of the various heaters) such that the current capacity of the high voltage power is not exceeded. Multiple heaters can be controlled by different PWM signal generators with varying start and end counts. The heaters can be divided into banks, whereby a bank defines a group of heaters of the same start count. For example, 36 PWM generators can be grouped into six different banks, each corresponding to a certain por-60 tion of the PWM cycle (500 ms for this example). The end count for each PWM generator can be selectively programmed such that not more than six heaters will be on at any given time. A portion of a PWM cycle can be selected as dead time (count 3000 to 4000 for this example) during which no heating takes place and sensitive temperature sensing circuits can use this time to sense the temperature. The table below represents a PWM cycle for the foregoing example:

#### Example 4

#### Detector Integrated in Force Member

embodiments of a detection system integrated into a force member, in an apparatus for carrying out diagnostics on microfluidic samples.

FIG. 43A: The lid of the apparatus can be closed, which can block ambient light from the sample bay, and place an optical 45 detector contained in the lid into position with respect to the microfluidic cartridge.

FIG. 43B: The lid of the apparatus can be closed to apply pressure to the cartridge. Application of minimal pressure on the cartridge: after the slider compresses the cartridge, the 50 slider can compress the compliant label of the cartridge. This can cause the bottom of the cartridge to be pressed down against the surface of the heater unit present in the heater module. Springs present in the slider can deliver, for example approximately 50 lb of pressure to generate a minimum pres- 55 sure, for example 2 psi over the entire cartridge bottom.

Thermal interface: the cartridge bottom can have a layer of mechanically compliant heat transfer laminate that can enable thermal contact between the microfluidic substrate and the microheater substrate of the heater module. A mini- 60 mal pressure of 1 psi can be employed for reliable operation of the thermal valves, gate and pumps present in the microfluidic cartridge.

Mechanicals and assembly: the Analyzer can have a simple mechanical frame to hold the various modules in alignment. 65 The optics module can be placed in rails for easy opening and placement of cartridges in the Analyzer and error-free align44

ment of the optics upon closing. The heater/sensor module can be also placed on rails or similar guiding members for easy removal and insertion of the assembly.

Slider: the slider of the Analyzer can house the optical detection system as well as the mechanical assembly that can enables the optics jig to press down on the cartridge when the handle of the slider is turned down onto the analyzer. The optics jig can be suspended from the case of the slider at 4 points. Upon closing the slider and turning the handle of the analyzer down, 4 cams can turn to push down a plate that presses on 4 springs. On compression, the springs can deliver approximately 50 lb on the optical block. See FIGS. 44A-44C.

The bottom surface of the optics block can be made flat to within 100 microns, typically within 25 microns, and this flat surface can press upon the compliant (shore hardness approximately 50-70) label (approximately 1.5 mm thick under no compression) of the cartridge making the pressure more or less uniform over the cartridge. An optional lock-in mechanism can also be incorporated to prevent the slider from being accidentally knocked-off while in use.

FIG. 45A shows a side view of a lever assembly 1200, with lever 1210, gear unit 1212, and force member 1214. Assem-25 bly 1200 can be used to close the lid of the apparatus and (through force members 1214) apply force to a microfluidic cartridge 1216 in the receiving chamber 1217. One force member is visible in this cut away view, but any number, for example 4, can be used. The force members can be, for example, a manual spring loaded actuator as shown, an automatic mechanical actuator, a material with sufficient mechanical compliance and stiffness (e.g., a hard elastomeric plug), and the like. The force applied to the microfluidic 35 cartridge 1216 can result in a pressure at the surface of the microfluidic cartridge 1216 of at least about 0.7 psi to about 7 psi (between about 5 and about 50 kilopascals), or in some embodiments about 2 psi (about 14 kilopascals.

FIG. 45B shows a side view of lever assembly 1200, with This non-limiting example describes pictorially, various 40 microfluidic cartridge 1216 in the receiving chamber 1217. A heat source 1219 (for example, a xenon bulb as shown) can function as a radiant heat source directed at a sample inlet reservoir 1218, where the heat can lyse cells in reservoir 1218. A thermally conductive, mechanically compliant layer 1222 can lie at an interface between microfluidic cartridge 1216 and thermal stage 1224. Typically, microfluidic cartridge 1216 and thermal stage 1224 can be planar at their respective interface surfaces, e.g., planar within about 100 microns, or more typically within about 25 microns. Layer 1222 can improve thermal coupling between microfluidic cartridge 1216 and thermal stage 1224. Optical detector elements 1220 can be directed at the top surface of microfluidic cartridge 1216.

FIGS. 45C and 45D show further cross-sectional views.

#### Example 6

#### **Exemplary Optics Board**

An exemplary optics board is shown schematically in FIG. 46, and is used to collect and amplify the fluorescent signature of a successful chemical reaction on a micro-fluidic cartridge, and control the intensity of LED's using pulse-width modulation (PWM) to illuminate the cartridge sample over up to four channels, each with two color options. Additionally, it receives instructions and sends results data back over an LVDS (low-voltage differential signaling) SPI (serial periph20

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eral interface). In some embodiments there is a separate instance of this circuitry for each PCR channel that is moni-

The power board systems include: a +12V input; and +3.3V, +3.6V, +5V, and -5V outputs, configured as follows: 5 the +3.3V output contains a linear regulator, is used to power the LVDS interface, should maintain a +1-5% accuracy, and supply an output current of 0.35 A; the +3.6V output contains a linear regulator, is used to power the MSP430, should maintain a  $\pm -5\%$  accuracy, and supply an output current of 0.35 A; 10 the +5V output contains a linear regulator, is used to power the plus rail for op-amps, should maintain a +1-5% accuracy, and supply an output current of 0.35 A; the -5V output receives its power from the +5V supply, has a mV reference, is used to power the minus rail for op-amps and for the 15 photo-detector bias, should maintain a +/-1% voltage accuracy, and supply an output current of 6.25 mA +/-10%. Additionally, the power board has an 80 ohm source resistance, and the main board software can enable/disable the regulator outputs.

The main board interface uses a single channel of the LVDS standard to communicate between boards. This takes place using SPI signaling over the LVDS interface which is connected to the main SPI port of the control processor. The interface also contains a serial port for in-system program- 25 ming.

The optical detection system of FIG. 46 comprises a control processor, LED drivers, and a photo-detection system. In the exemplary embodiment, the control processor is a TI MSP430F1611 consisting of a dual SPI (one for main board 30 interface, and one for ADC interface) and extended SRAM for data storage. It has the functions of power monitoring, PWM LED control, and SPI linking to the ADC and main board. The LED drivers contain NPN transistor switches, are connected to the PWM outputs of the control processor, can 35 sink 10 mA @ (12V per LED (80 mA total), and are single channel with 2 LEDs (one of each color) connected to each. The photo-detection system has two channels and consists of a photo-detector, high-sensitivity photo-diode detector, high gain current to voltage converter, unity gain voltage inverting 40 amplifier, and an ADC. Additionally it contains a 16 channel Sigma-delta (only utilizing the first 8 channels) which is connected to the second SPI port of the control processor.

During assembly of the various components on to the PC board, such as may occur on a production line, there are the 45 following considerations. The extremely high impedance of the photo-detection circuit means that a rigorous cleaning procedure must be employed. Such a procedure may include, for example: After surface mount components are installed, the boards are washed on a Weskleen and blow dried upon 50 exiting conveyor. The belt speed can be set at 20-30. The boards are soaked in an alcohol bath for approximately 3 minutes, then their entire top and bottom surfaces are scrubbed using a clean, soft bristle brush. The boards are baked in a  $105^{\circ}$  F.  $(40^{\circ}$  C.) oven for 30 minutes to dry out all 55 components.

After all the components are installed: the soldered areas of the boards can be hand wash using deionized water and a soft bristle brush. The same soldered areas can be hand washed using alcohol and a soft bristle brush. The boards are allowed 60 to air dry. Once the board is cleaned, the optical circuitry must be conformal coated to keep contaminates out.

The foregoing description is intended to illustrate various aspects of the present technology. It is not intended that the examples presented herein limit the scope of the present 65 technology. The technology now being fully described, it will be apparent to one of ordinary skill in the art that many

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changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed:

- 1. An apparatus, comprising:
- a multi-lane microfluidic cartridge, each lane comprising a PCR reaction zone;
- a receiving bay configured to receive the microfluidic cartridge;
- each PCR reaction zone comprising a separately controllable heat source thermally coupled thereto, wherein the heat source maintains a substantially uniform temperature throughout the PCR reaction zone and thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction
- a detector configured to detect the presence of an amplification product in the respective PCR reaction zone; and
- a processor coupled to the detector and the heat source, configured to control heating of one or more PCR reaction zones by the heat sources.
- 2. The apparatus of claim 1, further comprising a registration member that is complementary to the microfluidic cartridge, whereby the receiving bay receives the microfluidic cartridge in a single orientation.
- 3. The apparatus of claim 1, wherein the processor is programmable to operate the detector to detect a polynucleotide or a probe thereof in a microfluidic cartridge located in the receiving bay.
- 4. The apparatus of claim 3, wherein the detector is an optical detector.
- 5. The apparatus of claim 4, wherein the optical detector comprises a light source that selectively emits light in an absorption band of a fluorescent dye and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof.
- 6. The apparatus of claim 5, wherein the optical detector comprises a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye.
- 7. The apparatus of claim 6, wherein the optical detector is configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof.
- 8. The apparatus of claim 6, wherein the optical detector is configured to independently detect a plurality of fluorescent dyes at a plurality of different locations, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof.
- 9. The apparatus of claim 1, wherein the processor is programmable to operate the plurality of heat sources.
- 10. The apparatus of claim 1, wherein at least one of the plurality of heat sources is a contact heat source selected from a resistive heater, a radiator, a fluidic heat exchanger and a Peltier device.
- 11. The apparatus of claim 10, wherein the contact heat source is configured at the receiving bay to be thermally coupled to a distinct location in a microfluidic cartridge received in the receiving bay, whereby the distinct location is selectively heated.
- 12. The apparatus of claim 11, wherein the distinct location has a surface area of between about 1 mm<sup>2</sup> and about 225  $mm^2$

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- 13. The apparatus of claim 12, wherein the contact heat source is configured to be in direct physical contact with a distinct location of a microfluidic cartridge received in the receiving bay.
- 14. The apparatus of claim 12, further comprising a compliant layer at the contact heat source configured to thermally couple the contact heat source with at least a portion of a microfluidic cartridge received in the receiving bay.
- 15. The apparatus of claim 14, wherein the compliant layer at the contact heat source has a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100.
- 16. The apparatus of claim 11, wherein the distinct location has a surface area of between about 1 mm<sup>2</sup> and about 100 mm<sup>2</sup>.
- 17. The apparatus of claim 16, further comprising at least one additional contact heat source, wherein the contact heat sources are each configured at the receiving bay to be independently thermally coupled to a different distinct location in a microfluidic cartridge received in the receiving bay, whereby the distinct locations are independently heated.
- 18. The apparatus of claim 1, wherein at least one of the plurality of heat sources is a radiative heat source configured to direct heat to a distinct location of a microfluidic cartridge received in the receiving bay.
- 19. The apparatus of claim 1, further comprising a lid at the receiving bay, the lid being operable to at least partially exclude ambient light from the receiving bay.
- 20. The apparatus of claim 19, wherein the lid is a sliding  $_{30}$
- 21. The apparatus of claim 19, wherein the lid comprises the optical detector.
- 22. The apparatus of claim 19, wherein a major face of the lid at the receiving bay varies from planarity by less than about 100 micrometers.
- 23. The apparatus of claim 19, wherein the lid is configured to be removable from the apparatus.
- 24. The apparatus of claim 19, wherein the lid comprises a latching member.
- 25. The apparatus of claim 1, further comprising at least one input device coupled to the processor, the input device

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being selected from the group consisting of a keyboard, a touch-sensitive surface, a microphone, a hard disk drive, an optical disk drive, a serial connection, a parallel connection, a wireless network connection, a wired network connection and a mouse.

- 26. The apparatus of claim 1, further comprising at least one sample identifier coupled to the processor, the sample identifier being selected from an optical character reader, a bar code reader and a radio frequency tag reader.
- 27. The apparatus of claim 26, wherein the sample identifier is a handheld bar code reader.
- 28. The apparatus of claim 1, further comprising at least one output coupled to the processor, the output being selected from a display, a printer, a speaker, a serial connection, a parallel connection, a wireless network connection and a wired network connection.
- 29. The apparatus of claim 1, further comprising a heating stage configured to be removable from the apparatus wherein at least one of the plurality of heat sources is located in the heating stage.
- **30**. The apparatus of claim **1**, configured to carry out PCR on multiple polynucleotide-containing samples, and to detect presence of an amplification product in each of the samples.
- **31**. The apparatus of claim **1**, configured to carry out PCR 25 on 4, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, or 48 samples.
  - **32**. The apparatus of claim **1**, wherein the polynucleotide-containing sample is PCR-ready.
  - **33**. A method of carrying out PCR on a plurality of samples, the method comprising:
    - introducing the plurality of samples into a multi-lane microfluidic cartridge, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples;

moving the plurality of samples into the respective plurality of PCR reaction zones; and

amplifying polynucleotides contained with the plurality of samples in the PCR reaction zones while thermal cycling the PCR reaction zones, at least one PCR reaction zone separately thermally controllable from another PCR reaction zone.

: \* \* \* \*

#### UNITED STATES PATENT AND TRADEMARK OFFICE

### **CERTIFICATE OF CORRECTION**

PATENT NO. : 7,998,708 B2

APPLICATION NO. : 11/985577

DATED : August 16, 2011

INVENTOR(S) : Handique et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In column 10 at line 4, change "dispsy" to --display--.

In column 10 at line 13, change "from" to --from,--.

In column 21 at line 66, change " $500\mu m$ " to --~ $500\mu m$ )--.

In column 21 at line 67, change " $500\mu$ m" to --~ $500\mu$ m)--.

In column 27 at line 48, change "melaminogenica;" to --melaninogenica;--.

In column 28 at line 55, change "Lactobacillus fermentum," to --Lactobacillus fermentum,--.

In column 34 at line 44, change "(C)." to --(C)--.

In column 36 at line 48, change " $G\Omega$ )." to -- $G\Omega$ .--.

In column 45 at line 7, change "+1-5%" to --+/-5%--.

In column 45 at line 12, change "+1-5%" to --+/-5%--.

In column 45 at line 36, change "(12V" to --12V--.

Signed and Sealed this Ninth Day of October, 2012

David J. Kappos

Director of the United States Patent and Trademark Office

# **EXHIBIT 4**

## (12) United States Patent

#### Handique et al.

#### (10) **Patent No.:**

## US 8,323,900 B2

#### (45) **Date of Patent:**

\*Dec. 4, 2012

#### (54) MICROFLUIDIC SYSTEM FOR AMPLIFYING AND DETECTING POLYNUCLEOTIDES IN PARALLEL

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(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-

claimer.

(21) Appl. No.: 13/035,725

(22) Filed: Feb. 25, 2011

#### (65) **Prior Publication Data**

US 2011/0207140 A1 Aug. 25, 2011

#### Related U.S. Application Data

- (63) Continuation of application No. 11/985,577, filed on Nov. 14, 2007, now Pat. No. 7,998,708, which is a continuation-in-part of application No. 11/728,964, filed on Mar. 26, 2007.
- (60) Provisional application No. 60/786,007, filed on Mar. 24, 2006, provisional application No. 60/859,284, filed on Nov. 14, 2006, provisional application No. 60/959,437, filed on Jul. 13, 2007.
- (51) **Int. Cl.**  *C12Q 1/68* (2006.01) *C12P 19/34* (2006.01)
- (52) **U.S. Cl.** ...... 435/6.12; 435/6.1; 435/6.11

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#### (57) ABSTRACT

The present technology provides for an apparatus for detecting polynucleotides in samples, particularly from biological samples. The technology more particularly relates to microfluidic systems that carry out PCR on nucleotides of interest within microfluidic channels, and detect those nucleotides. The apparatus includes a microfluidic cartridge that is configured to accept a plurality of samples, and which can carry out PCR on each sample individually, or a group of, or all of the plurality of samples simultaneously.

#### 22 Claims, 61 Drawing Sheets

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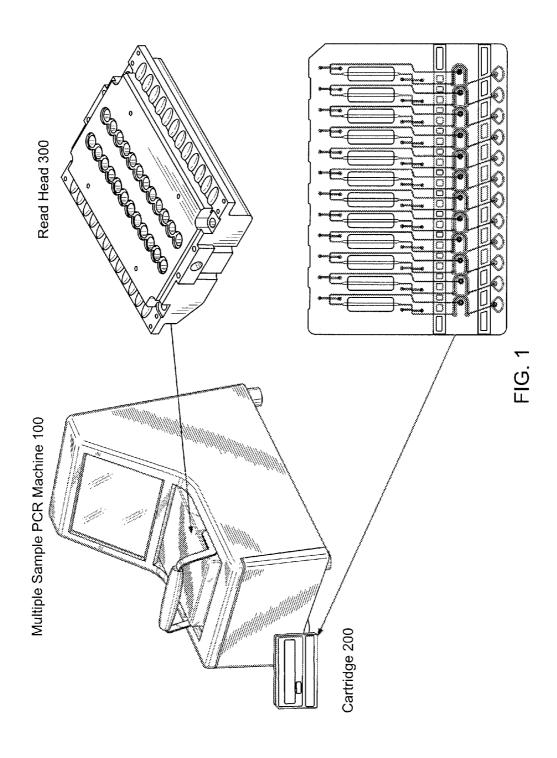
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## Sample Preparation Kit

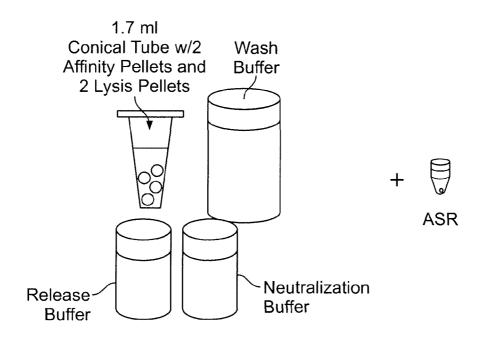


FIG. 2

Automated Dispensing Head

Cover for Optics

310

Cartridge
200

Heat
Substrate
400

Read Head

FIG. 4

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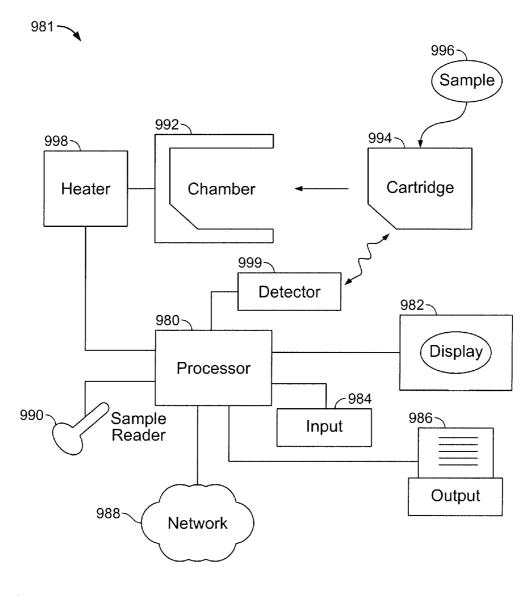


FIG. 3

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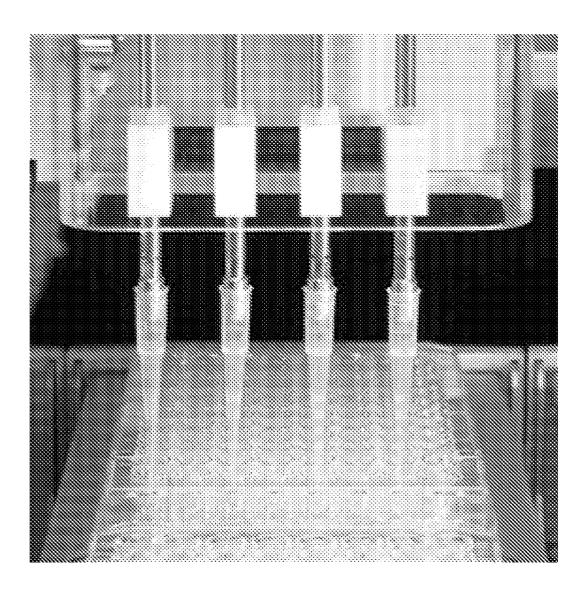


FIG. 5

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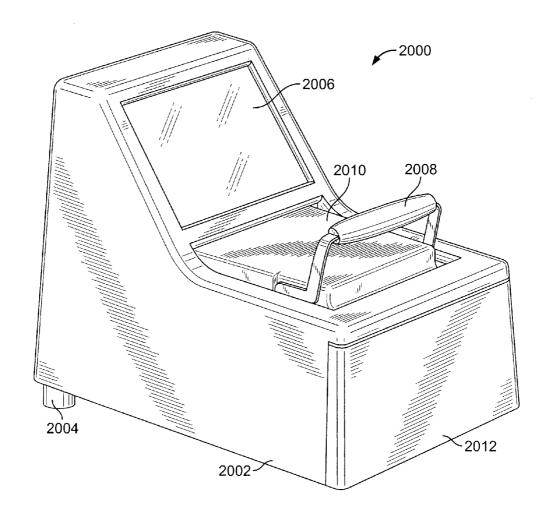


FIG. 6A

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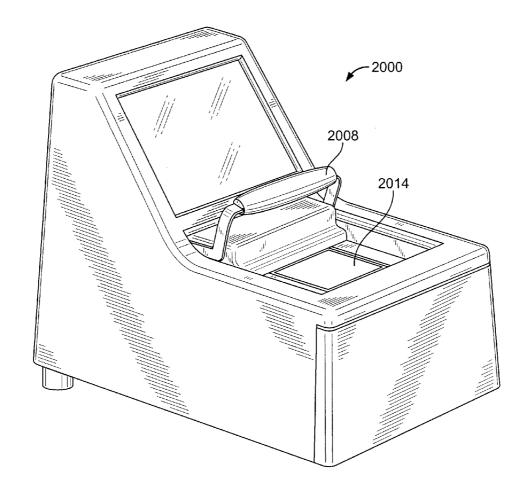


FIG. 6B

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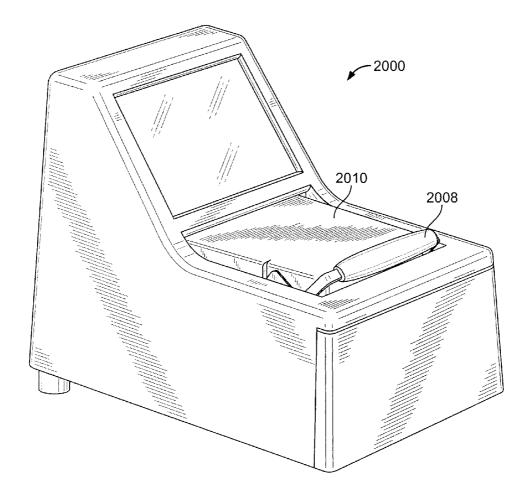
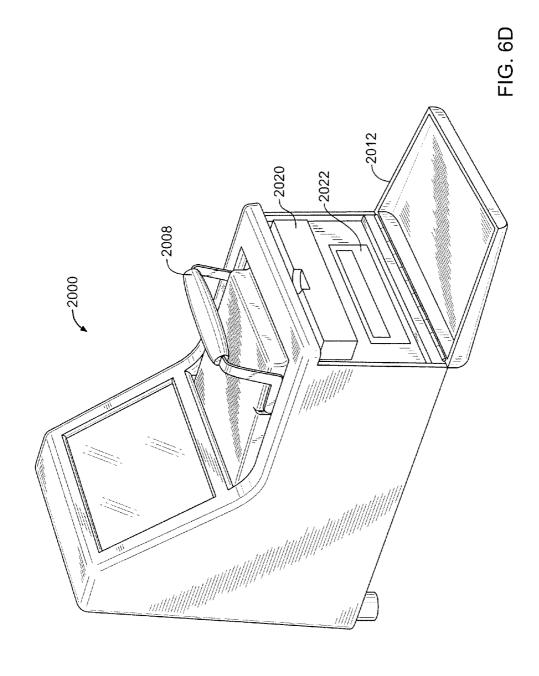


FIG. 6C

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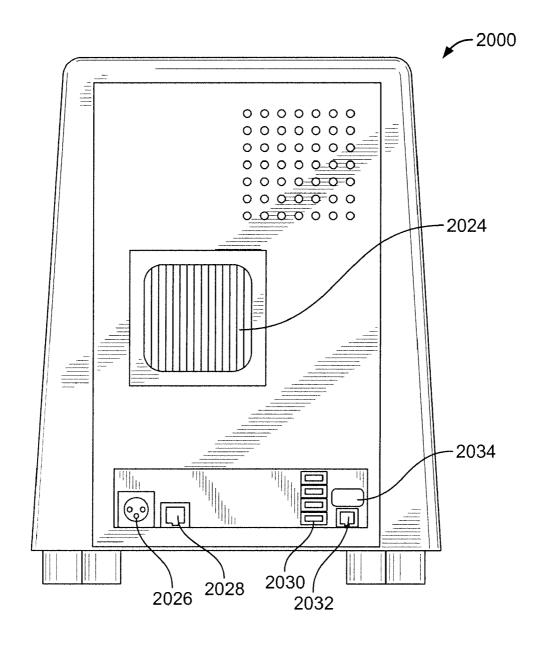


FIG. 6E

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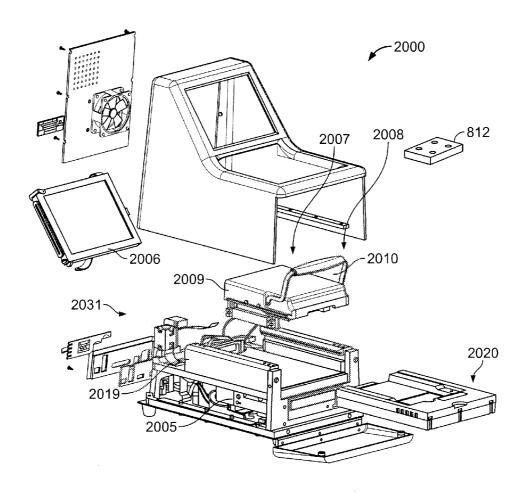
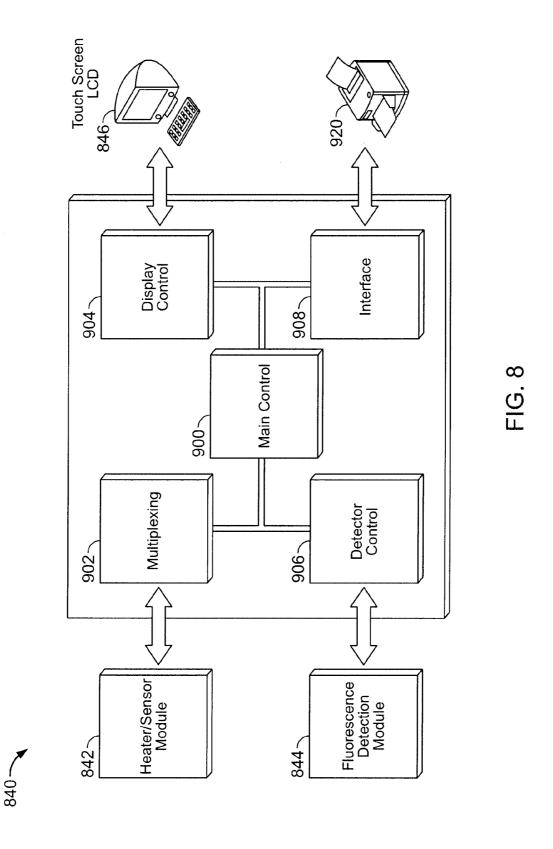


FIG. 7

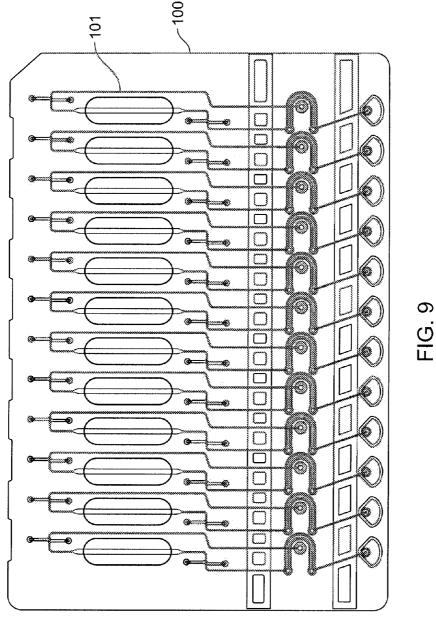
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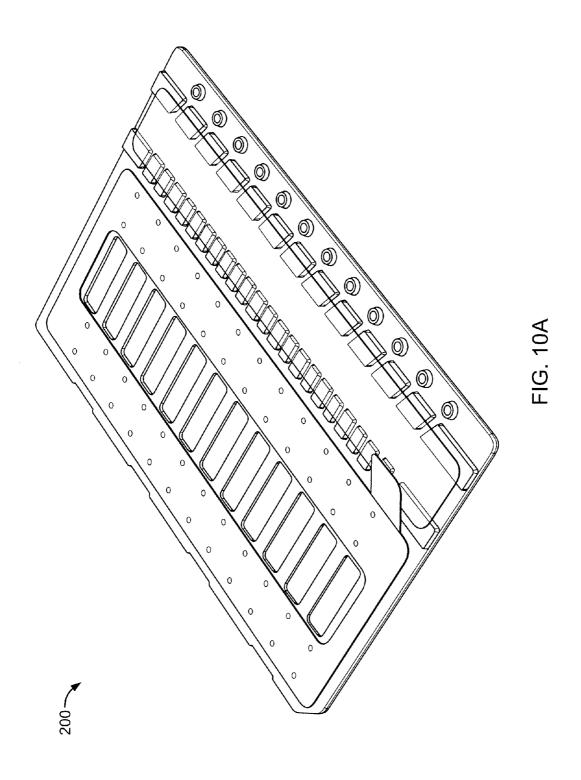
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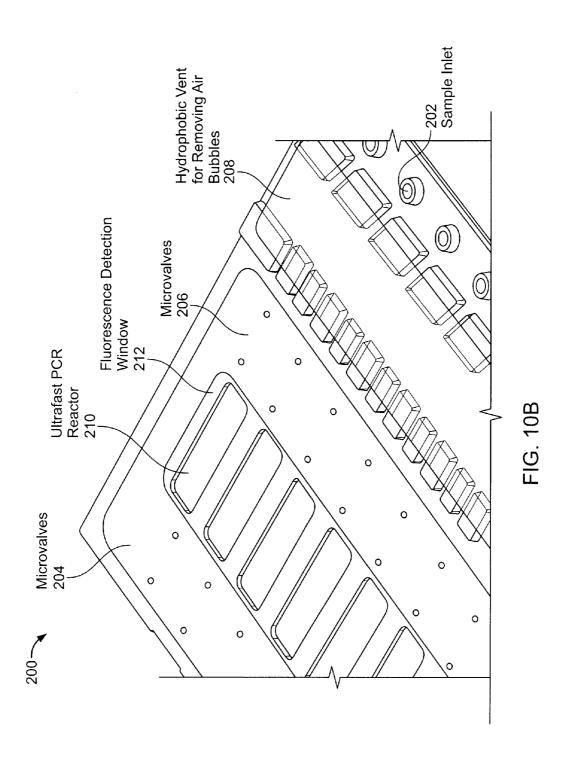
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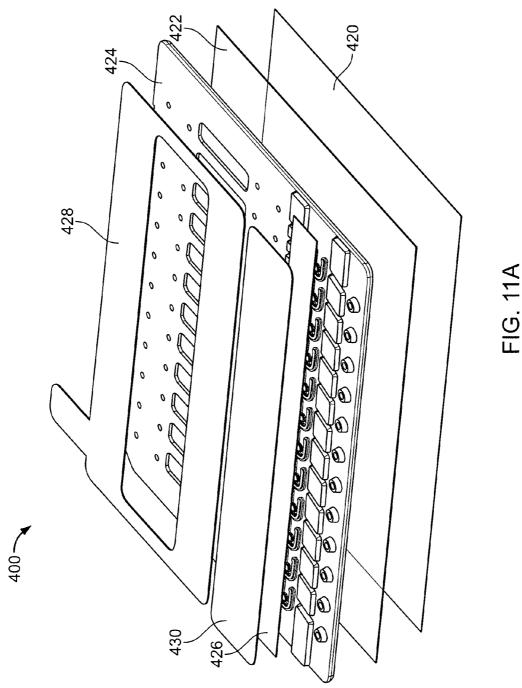
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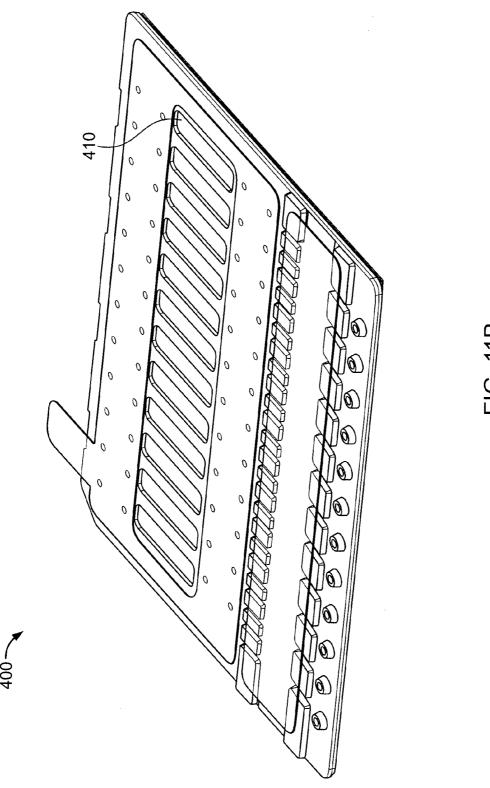


FIG. 11B

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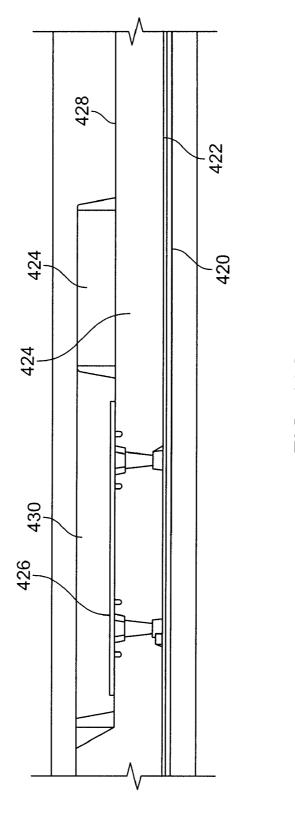
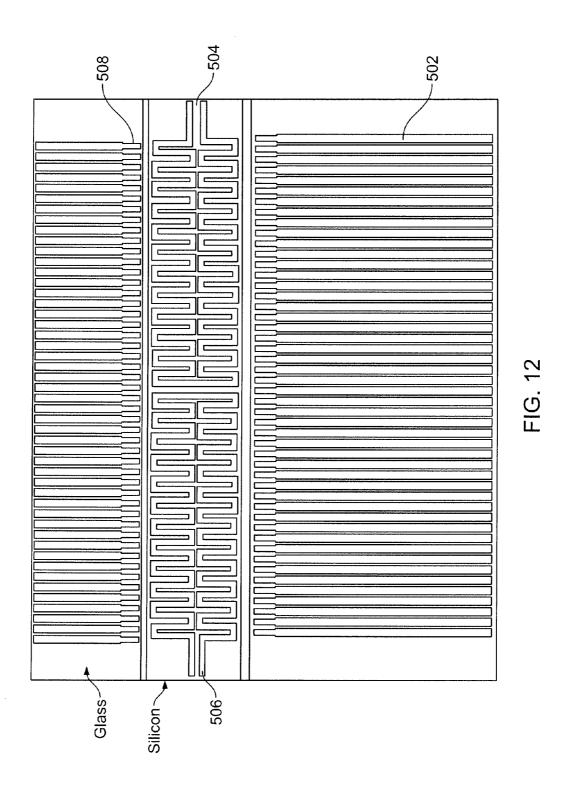


FIG. 11C

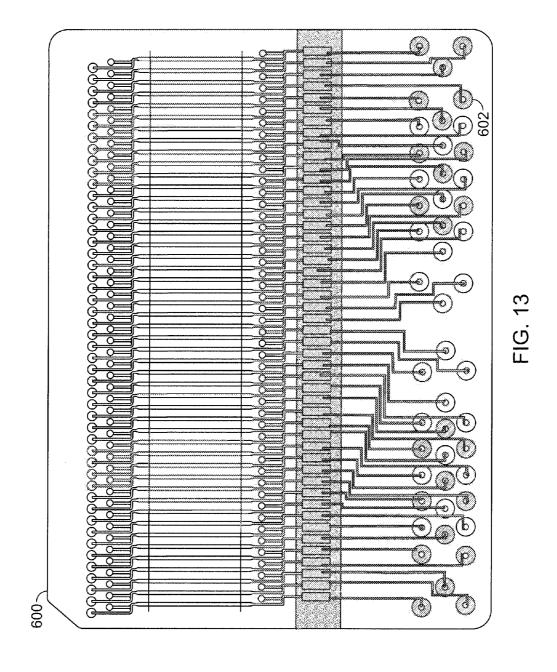
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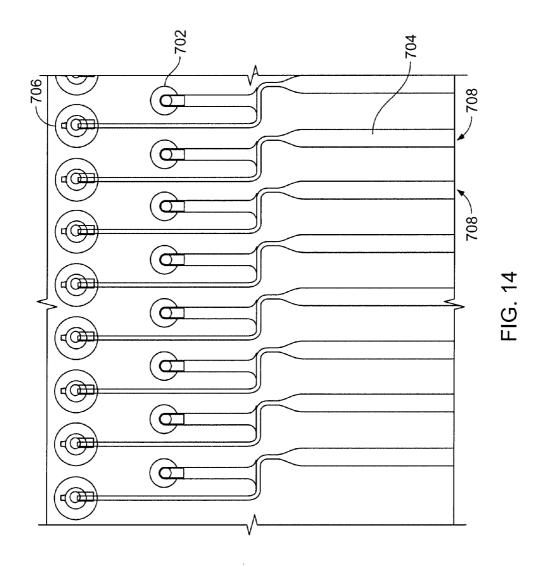
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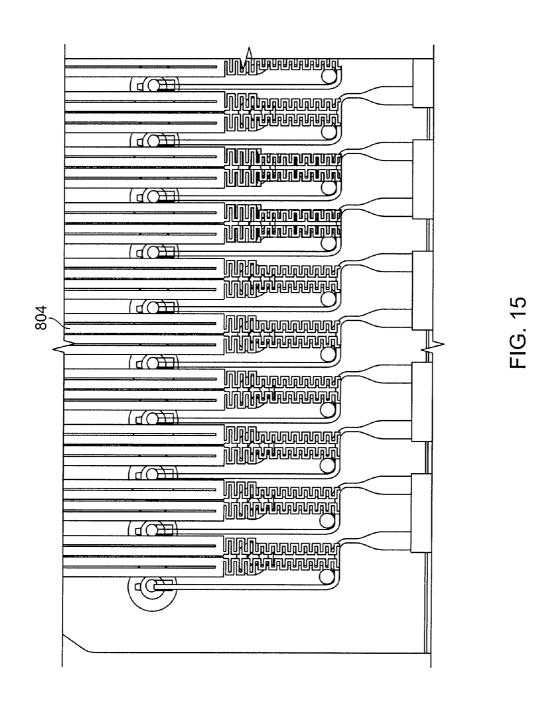
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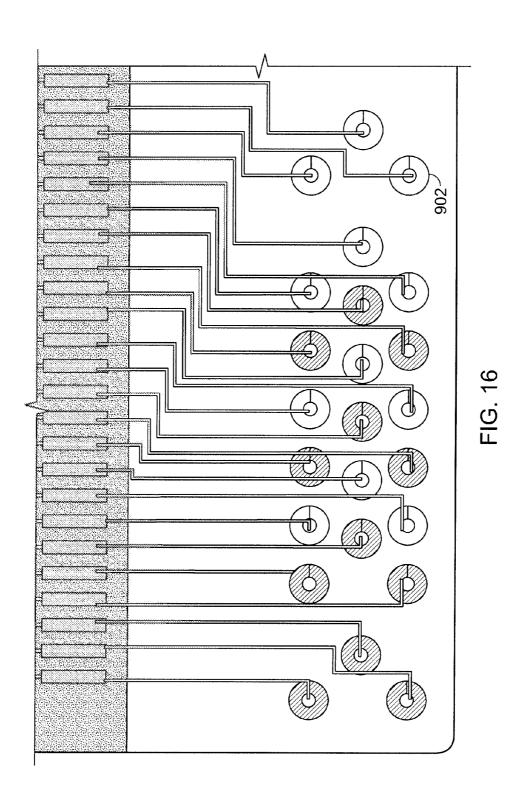
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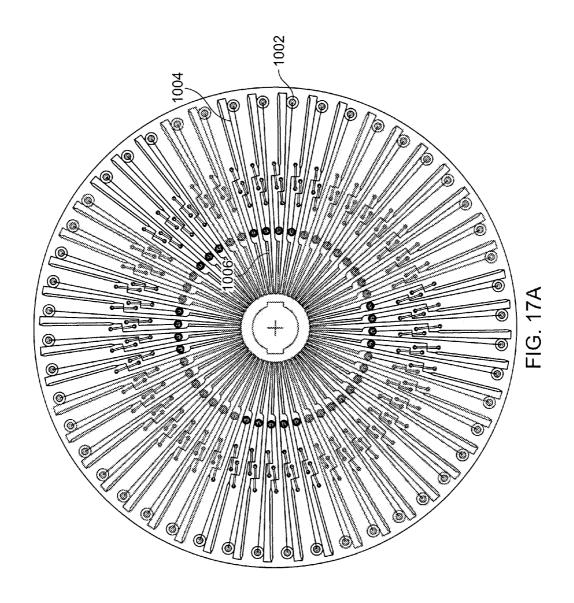
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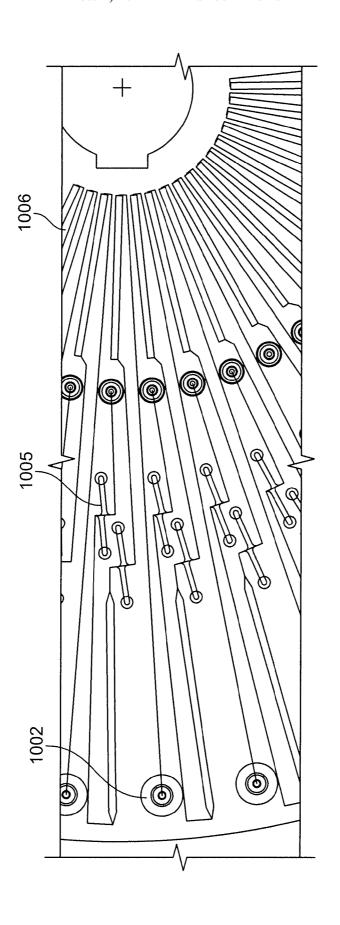
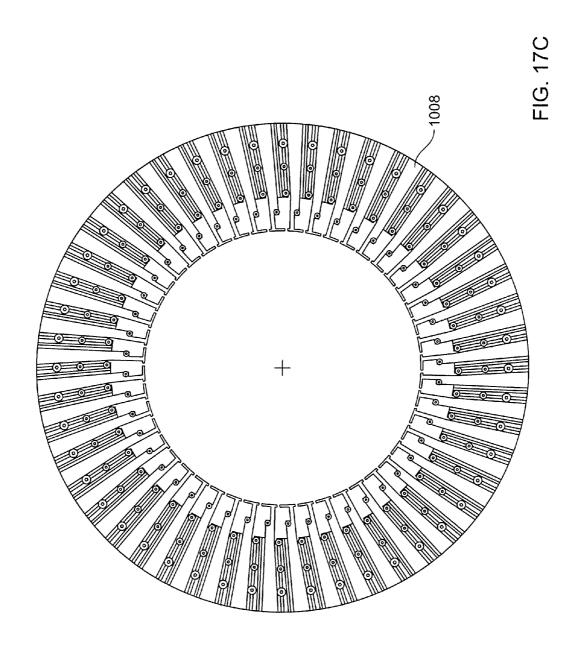


FIG. 17E

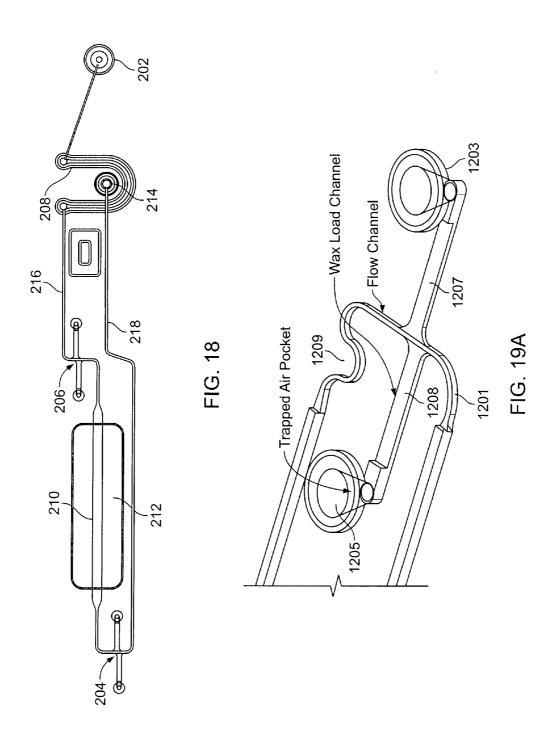
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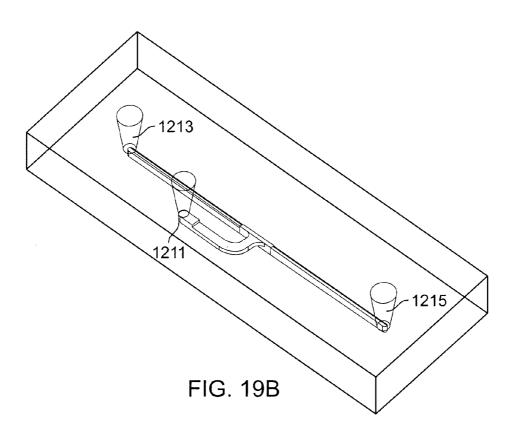
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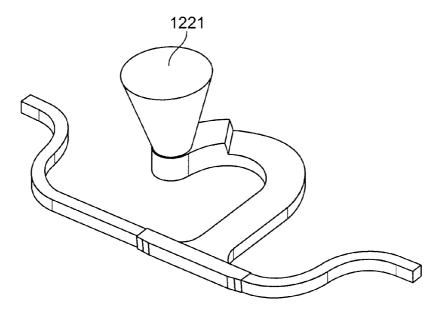
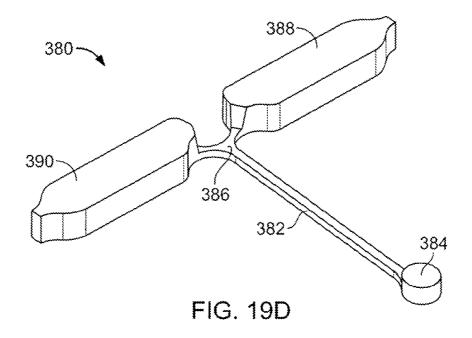


FIG. 19C

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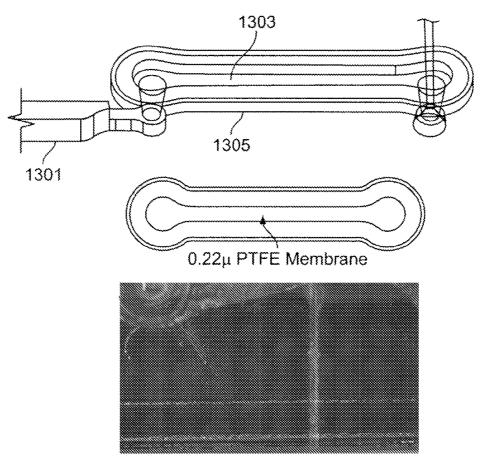
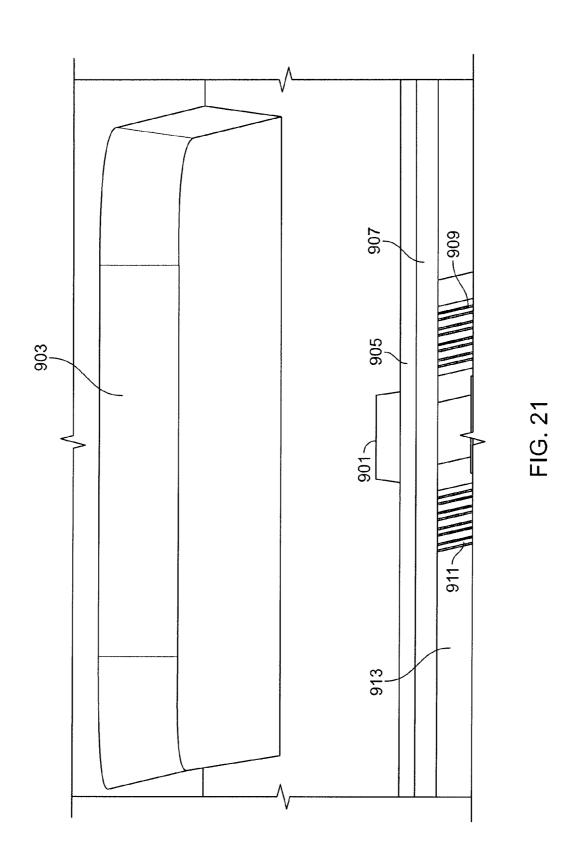


FIG. 20

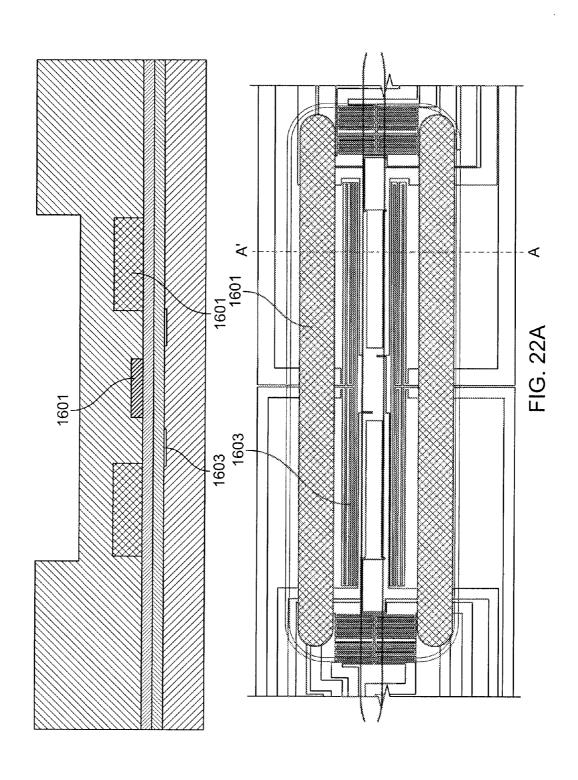
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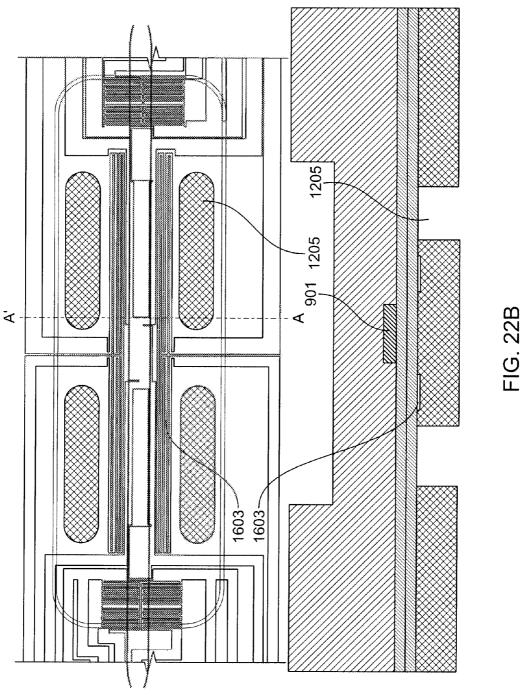
Dec. 4, 2012

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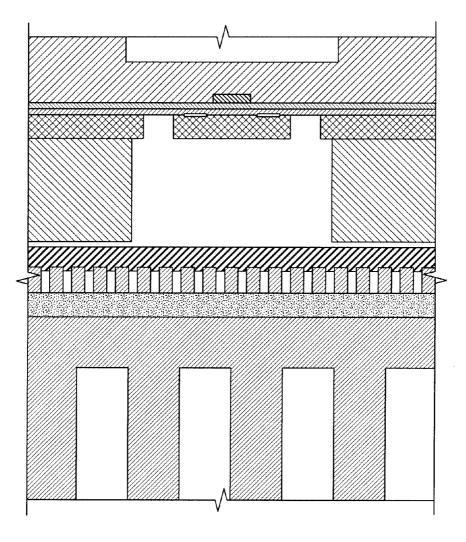
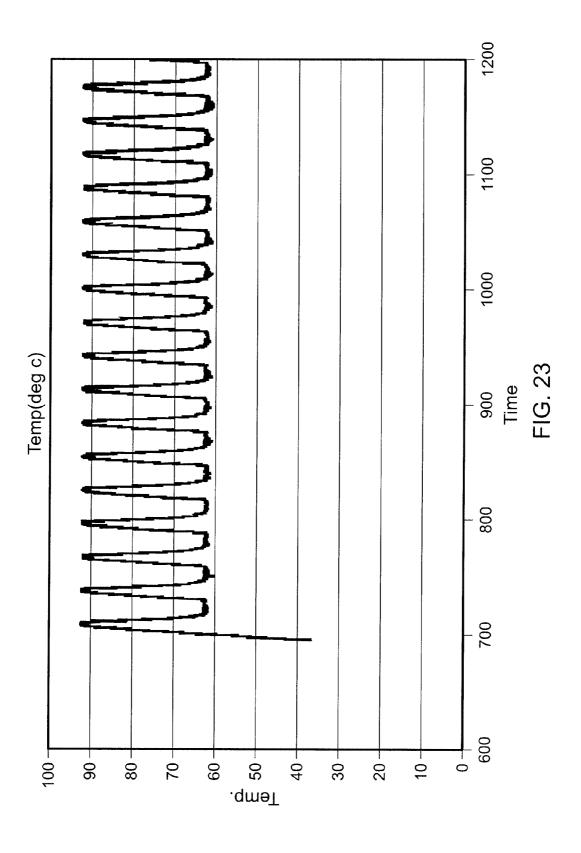


FIG. 22C

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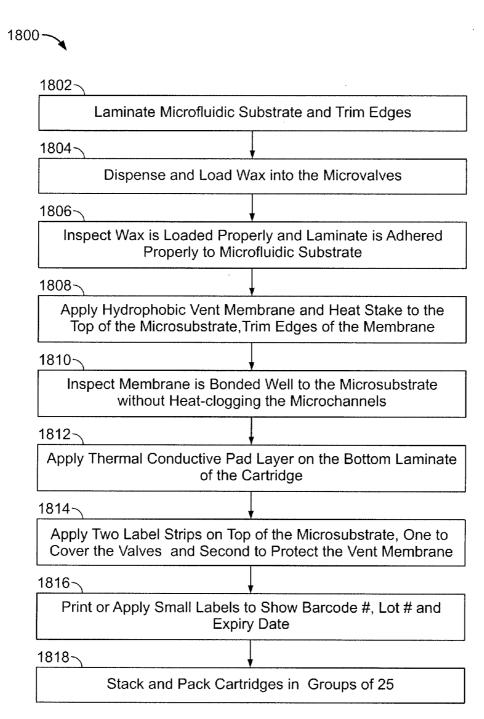


FIG. 24

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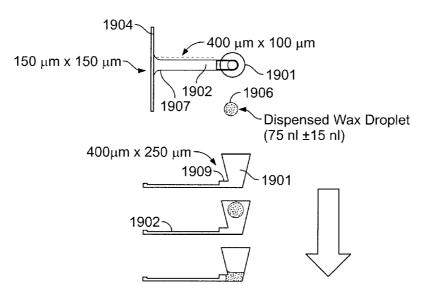
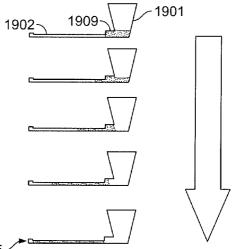


FIG. 25A

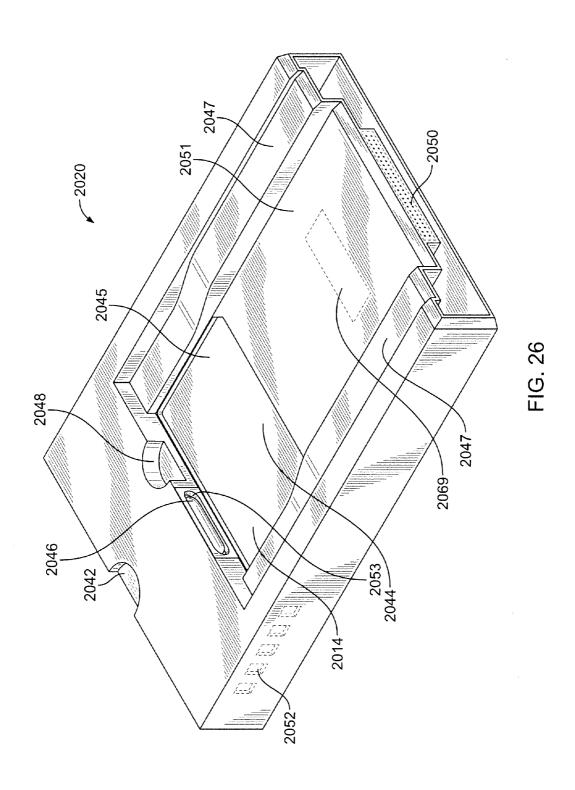


Capillary action of controlled volume of wax causes it to fill up the Wax up to the right interface without blocking the liquid flowable microchannel

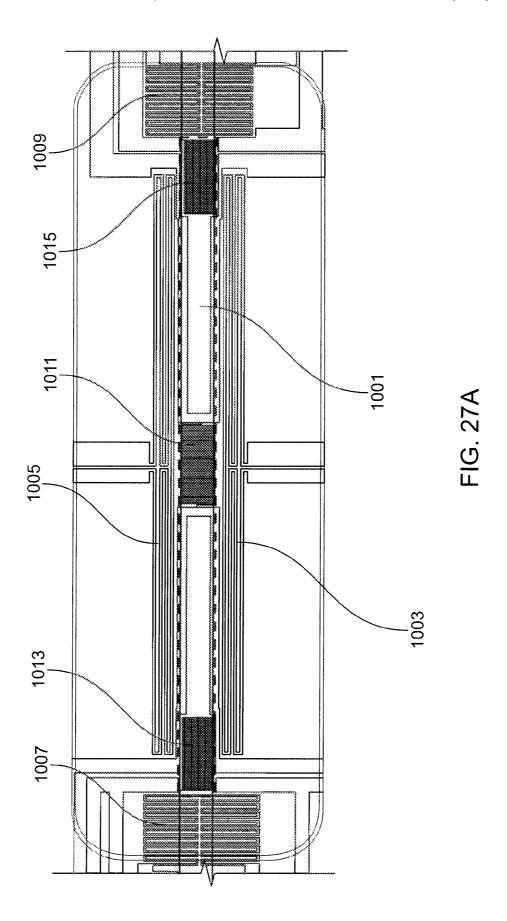
FIG. 25B

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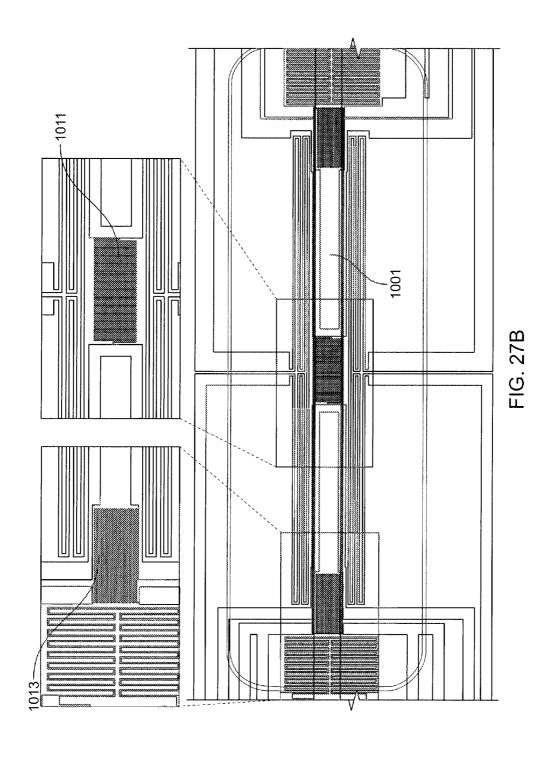


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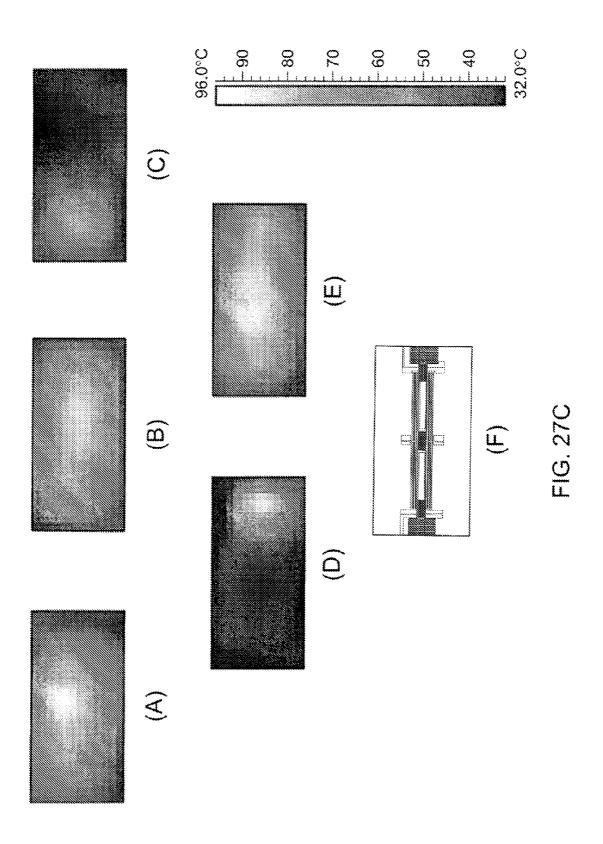
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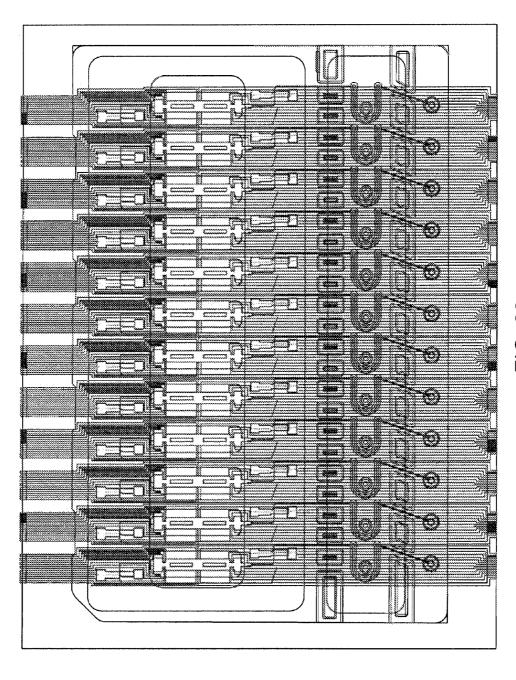


FIG. 28

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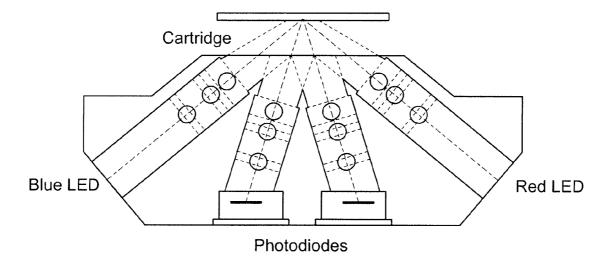
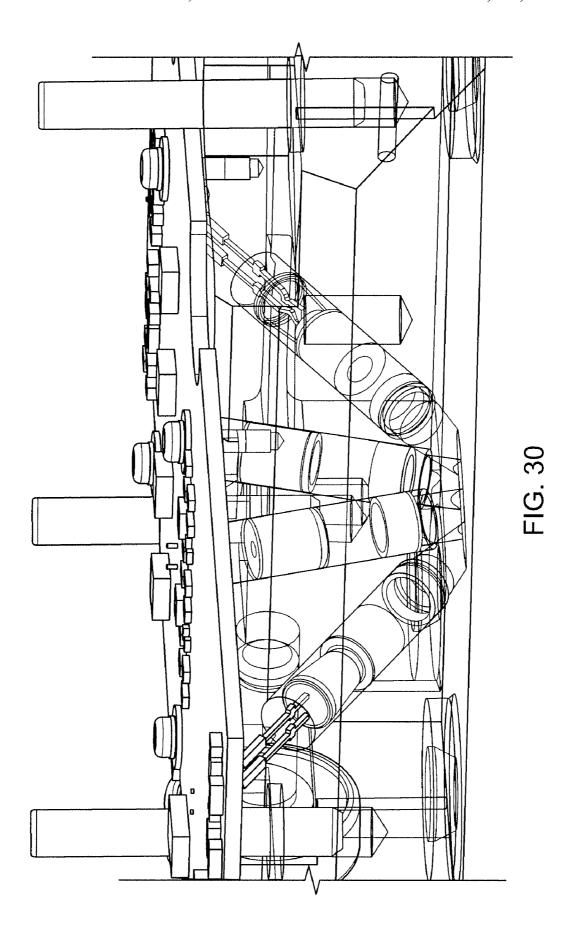


FIG. 29

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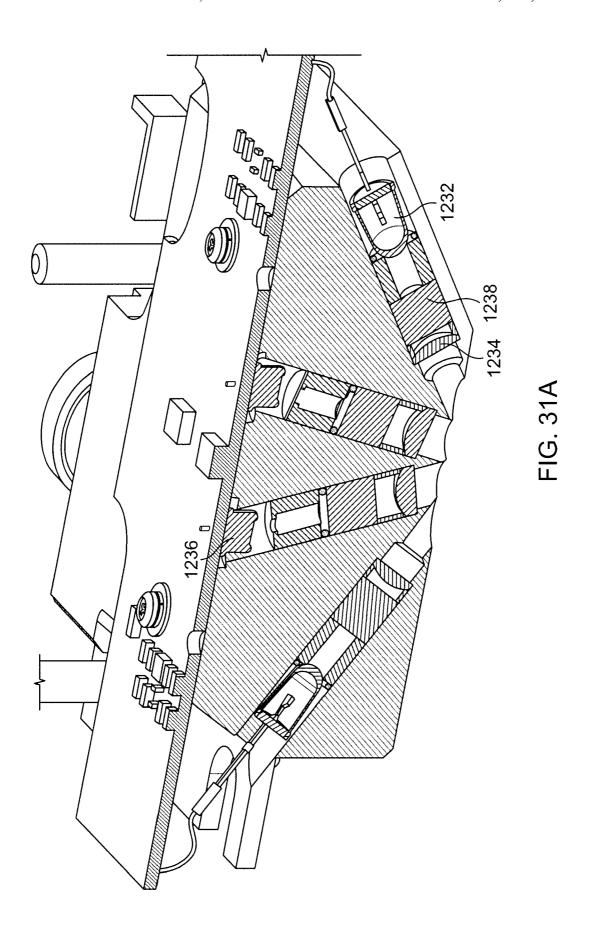
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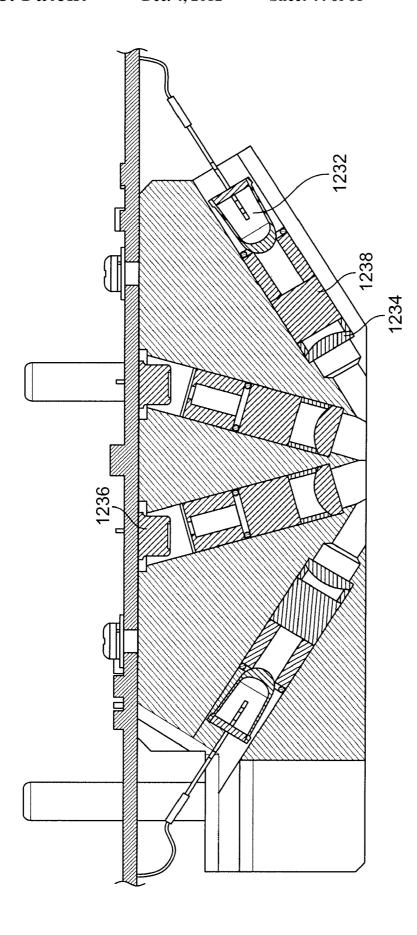


FIG. 31B

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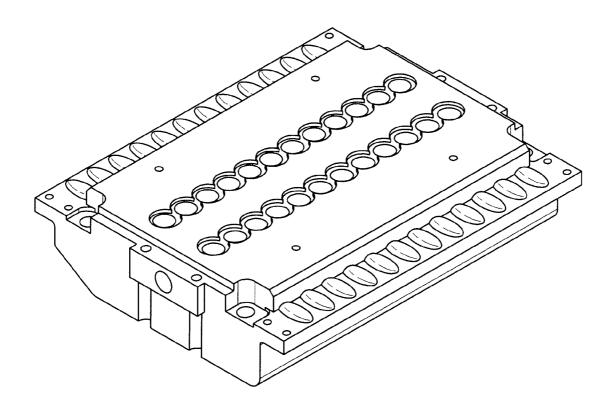


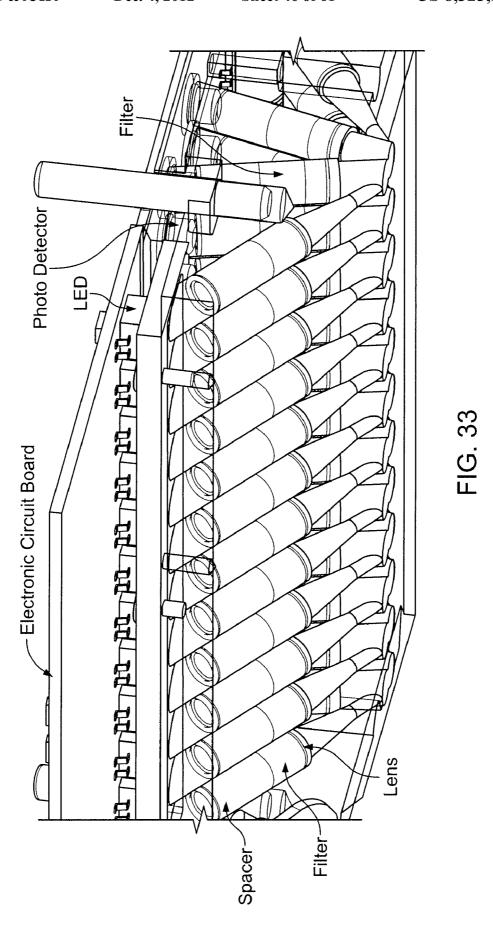
FIG. 32

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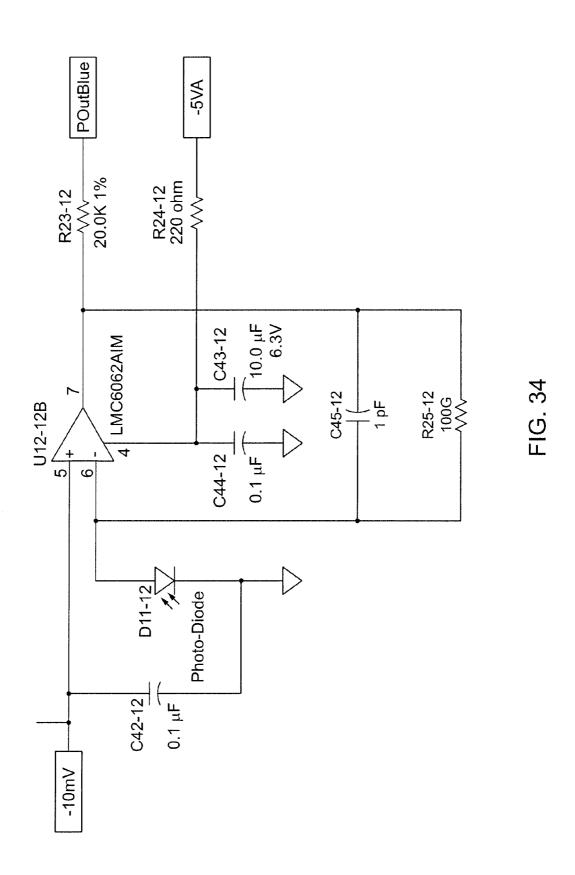
**Sheet 46 of 61** 

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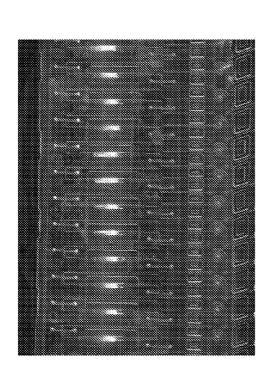
Dec. 4, 2012

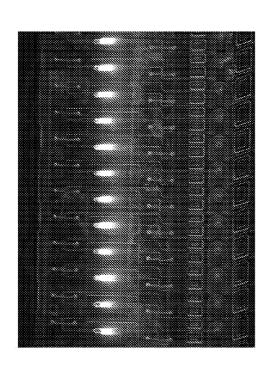
**Sheet 47 of 61** 

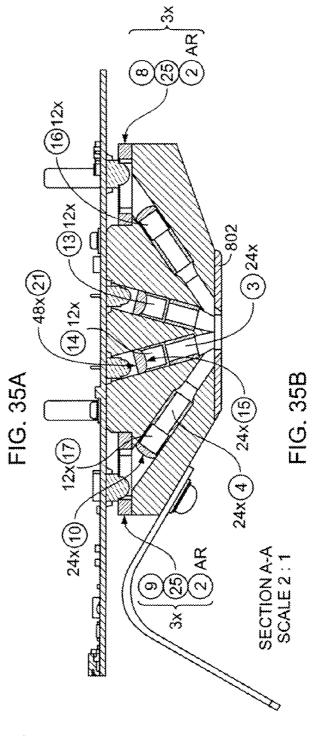


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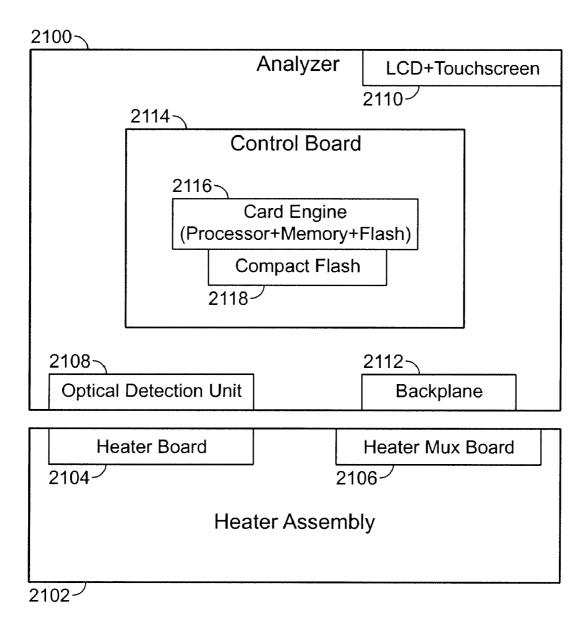


FIG. 36

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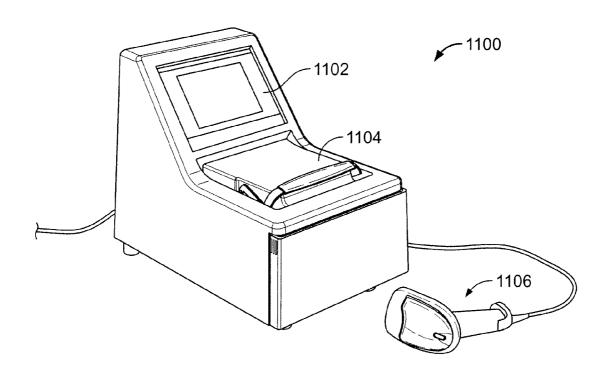


FIG. 37

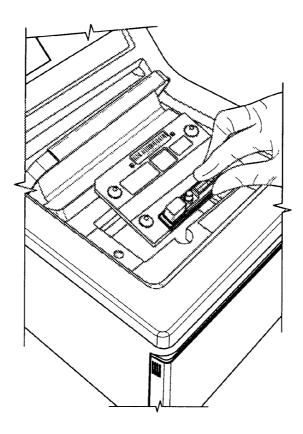
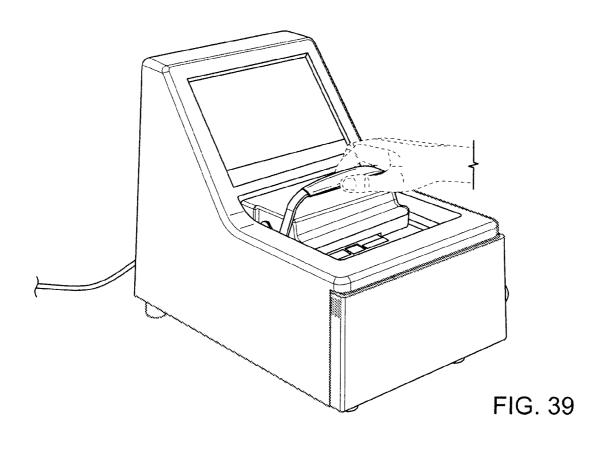


FIG. 38

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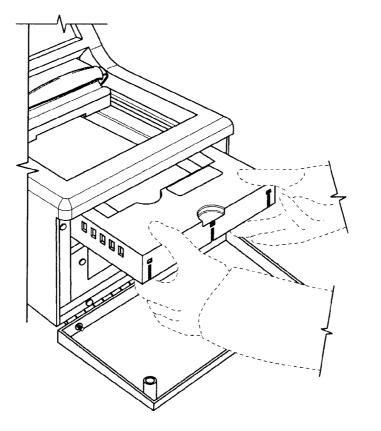


FIG. 40

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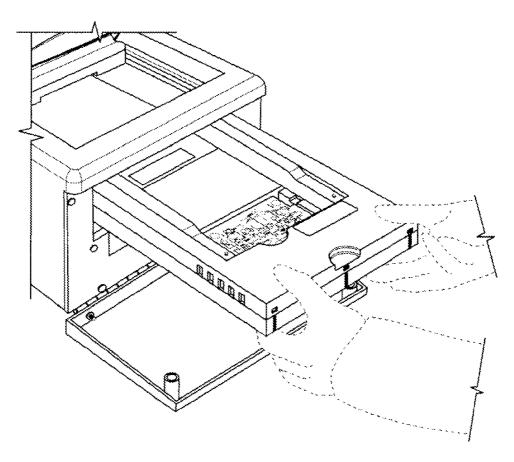


FIG. 41

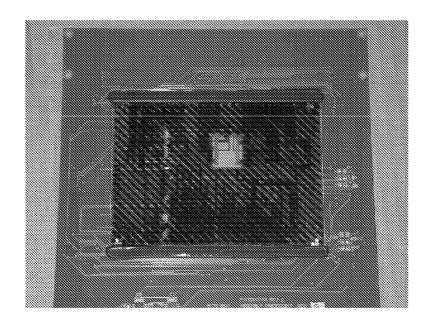


FIG. 42B

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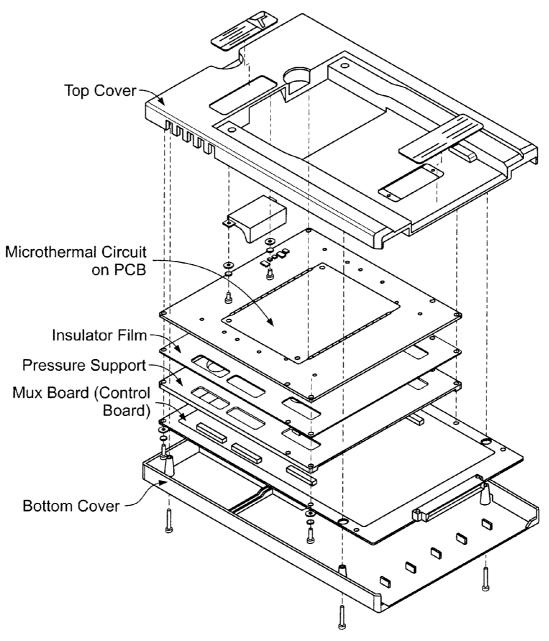
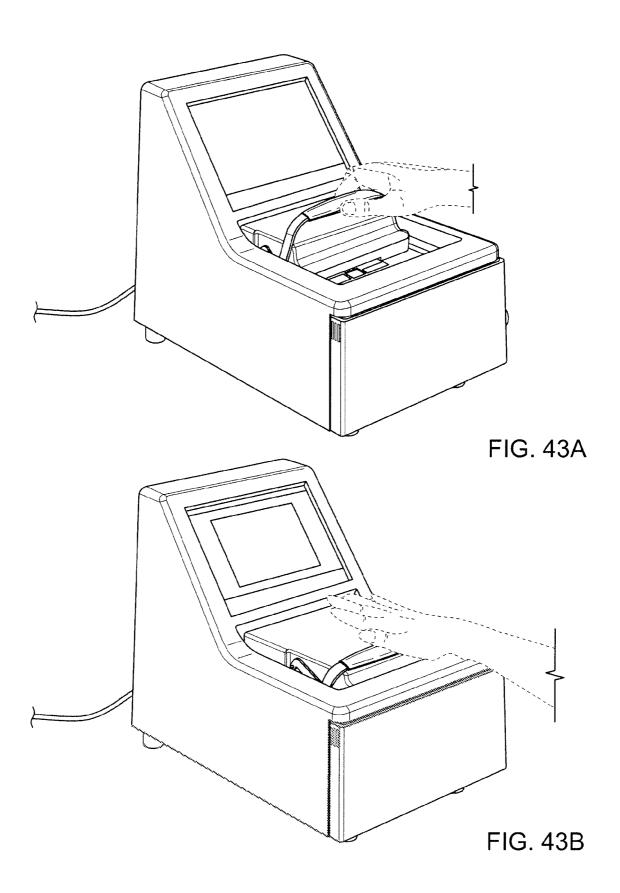


FIG. 42A

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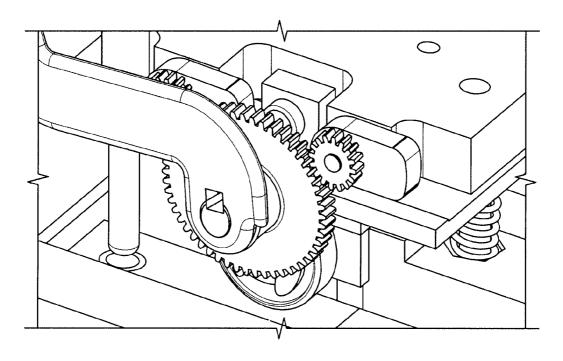


FIG. 44A

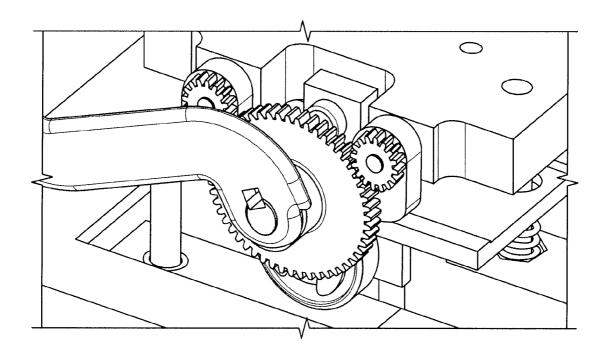


FIG. 44B

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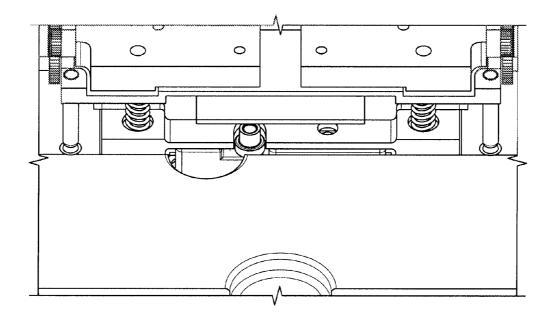
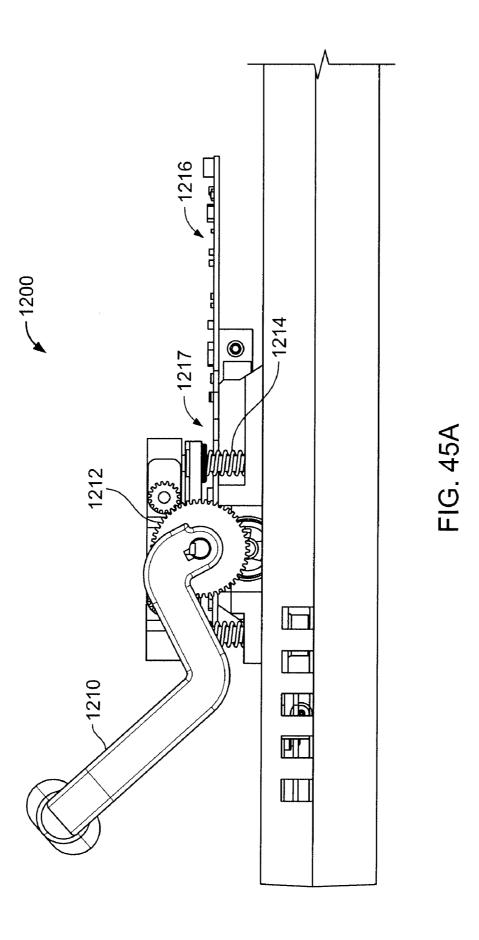
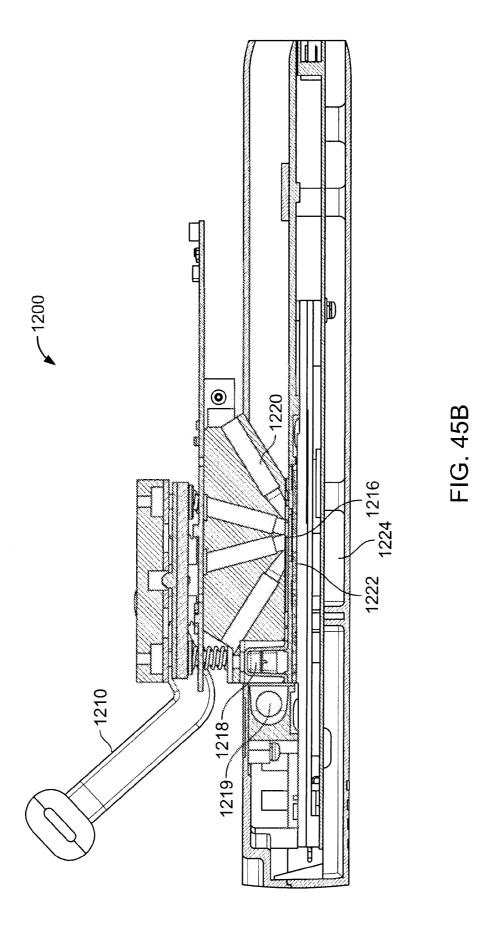


FIG. 44C

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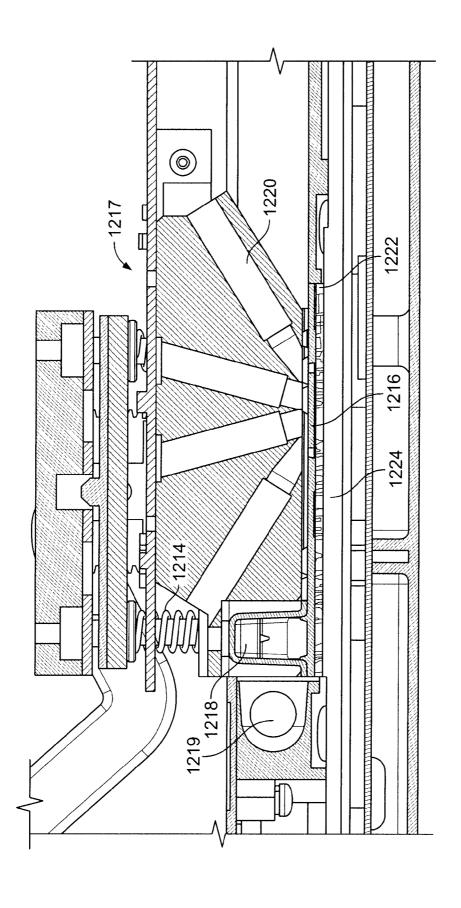


FIG. 450

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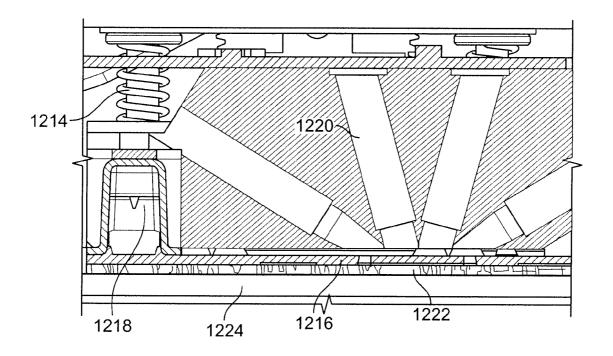


FIG. 45D

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FIG. 46

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#### MICROFLUIDIC SYSTEM FOR AMPLIFYING AND DETECTING POLYNUCLEOTIDES IN PARALLEL

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 11/985,577, filed Nov. 14, 2007, now U.S. Pat. No. 7,998,708 which is a continuation-in-part of U.S. patent application Ser. No. 11/728,964, filed Mar. 26, 2007, which claims the benefit of U.S. Provisional Patent Application No. 60/786,007, filed Mar. 24, 2006, and U.S. Provisional Patent Application No. 60/859,284, filed Nov. 14, 2006. U.S. patent 15 application Ser. No. 11/985,577 claims the benefit of U.S. Provisional Patent Application No. 60/859,284, filed Nov. 14, 2006, and U.S. Provisional Patent Application No. 60/959, 437, filed Jul. 13, 2007. The disclosures of U.S. patent application Ser. No. 11/985,577, U.S. patent application Ser. No. 20 11/728,964, U.S. Provisional Patent Application No. 60/859, 284, and U.S. Provisional Patent Application No. 60/959,437 are considered part of the disclosure of this application, and are incorporated by reference herein in their entirety.

#### TECHNICAL FIELD

The technology described herein generally relates to systems for detecting polynucleotides in samples, particularly from biological samples. The technology more particularly relates to microfluidic systems that carry out PCR on nucleotides of interest within microfluidic channels, and detect those nucleotides.

#### **BACKGROUND**

The medical diagnostics industry is a critical element of today's healthcare infrastructure. At present, however, diagnostic analyses no matter how routine have become a bottleneck in patient care. There are several reasons for this. First, 40 many diagnostic analyses can only be done with highly specialist equipment that is both expensive and only operable by trained clinicians. Such equipment is found in only a few locations—often just one in any given urban area. This means that most hospitals are required to send out samples for analyses to these locations, thereby incurring shipping costs and transportation delays, and possibly even sample loss. Second, the equipment in question is typically not available 'on-demand' but instead runs in batches, thereby delaying the processing time for many samples because they must wait for a 50 machine to fill up before they can be run.

Understanding that sample flow breaks down into several key steps, it would be desirable to consider ways to automate as many of these as possible. For example, a biological sample, once extracted from a patient, must be put in a form 55 suitable for a processing regime that typically involves using PCR to amplify a vector of interest. Once amplified, the presence of a nucleotide of interest from the sample needs to be determined unambiguously. Sample preparation is a process that is susceptible to automation but is also relatively 60 routinely carried out in almost any location. By contrast, steps such as PCR and nucleotide detection have customarily only been within the compass of specially trained individuals having access to specialist equipment.

There is a need for a method and apparatus of carrying out 65 PCR and detection on prepared biological samples, and preferably with high throughput. In particular there is a need for

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an easy-to-use device that can deliver a diagnostic result on several samples in a short time.

The discussion of the background to the technology herein is included to explain the context of the technology. This is not to be taken as an admission that any of the material referred to was published, known, or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word "comprise" and variations thereof, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

#### **SUMMARY**

The present technology addresses systems for detecting polynucleotides in samples, particularly from biological samples. In particular, the technology relates to microfluidic systems that carry out PCR on nucleotides of interest within microfluidic channels, and detect those nucleotides.

20 An apparatus, comprising: a receiving bay configured to receive a microfluidic cartridge; at least one heat source thermally coupled to the cartridge and configured to carry out PCR on a microdroplet of polynucleotide-containing sample, in the cartridge; a detector configured to detect presence of one or more polynucleotides in the sample; and a processor coupled to the detector and the heat source, configured to control heating of one or more regions of the microfluidic cartridge.

A method of carrying out PCR on a plurality of polynucleotide-containing samples, the method comprising: introducing the plurality of samples in to a microfluidic cartridge,
wherein the cartridge has a plurality of PCR reaction chambers configured to permit thermal cycling of the plurality of
samples independently of one another; moving the plurality
of samples into the respective plurality of PCR reaction
chambers; and amplifying polynucleotides contained with
the plurality of samples, by application of successive heating
and cooling cycles to the PCR reaction chambers.

The details of one or more embodiments of the technology are set forth in the accompanying drawings and further description herein. Other features, objects, and advantages of the technology will be apparent from the description and drawings, and from the claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an exemplary apparatus, a microfluidic cartridge, and a read head, as further described herein;

FIG. 2 shows an exemplary sample-preparation kit;

FIG. 3 shows a schematic diagram of an apparatus;

FIG. 4 shows a cross-section of a pipetting head and a cartridge in position in a microfluidic apparatus.

FIG. 5 shows introduction of a PCR-ready sample into a cartridge, situated in an instrument;

FIGS. **6**A-**6**E show exemplary embodiments of an apparatus;

FIG. 7 shows an exploded view of an apparatus;

FIG. 8 shows a block diagram of control circuitry;

FIG. **9** shows a plan view of an exemplary multi-lane 60 microfluidic cartridge;

FIG. 10A shows an exemplary multi-lane cartridge;

FIG. 10B shows a portion of an exemplary multi-lane cartridge;

FIGS. 11A-C show exploded view of an exemplary microfluidic cartridge;

FIG. 12 shows an exemplary highly-multiplexed microfluidic cartridge;

FIGS. 13-16 show various aspects of exemplary highly multiplexed microfluidic cartridges; and

FIGS. 17A-C show various aspects of a radially configured highly multiplexed microfluidic cartridge.

FIG. 18 shows an exemplary microfluidic network in a lane 5 of a multi-lane cartridge;

FIGS. 19A-19D show exemplary microfluidic valves:

FIG. 20 shows an exemplary bubble vent;

FIG. 21 shows a cross-section of a microfluidic cartridge, when in contact with a heater substrate;

FIGS. 22A-22C shows various cut-away sections that can be used to improve cooling rates during PCR thermal cycling;

FIG. 23 shows a plot of temperature against time during a PCR process, as performed on a microfluidic cartridge as 15 described herein;

FIG. 24 shows an assembly process for a cartridge as further described herein:

FIGS. 25A and 25B show exemplary deposition of wax droplets into microfluidic valves;

FIG. 26 shows an exemplary heater unit;

FIGS. 27A and 27B show a plan view of heater circuitry adjacent to a PCR reaction chamber;

FIG. 27C shows thermal images of heater circuitry in

FIG. 28 shows an overlay of an array of heater elements on an exemplary multi-lane microfluidic cartridge, wherein various microfluidic networks are visible;

FIG. 29 shows a cross-sectional view of an exemplary 30 detector;

FIG. 30 shows a perspective view of a detector in a read-

FIG. 31A, 31B shows a cutaway view of an exemplary detector in a read-head;

FIG. 32 shows an exterior view of an exemplary multiplexed read-head with an array of detectors therein;

FIG. 33 shows a cutaway view of an exemplary multiplexed read-head, as in FIG. 18;

FIG. 34 shows exemplary pre-amplifier circuitry for a fluorescence detector:

FIG. 35A shows effects of aperturing on fluorescence intensity; FIG. 35B shows a detector in cross section with an exemplary aperture;

FIG. 36 shows an exemplary layout for electronics and software components, as further described herein;

FIG. 37 shows an exemplary apparatus, a microfluidic cartridge, and a read head, as further described herein;

plary apparatus;

FIGS. 40 and 41 show removal of a heater unit from an exemplary apparatus;

FIGS. 42A and 42B show an exemplary heater unit and heater substrate;

FIGS. 43A and 43B show an exemplary apparatus having a detector mounted in a sliding lid;

FIGS. 44A-44C show a force member;

FIGS. 45A-45D show a force member associated with a  $_{60}$ detector;

FIG. 46 shows a block diagram of exemplary electronic circuitry in conjunction with a detector as described herein;

Additional figures are illustrated within the examples, and are further described therein.

Like reference symbols in the various drawings indicate like elements.

#### DETAILED DESCRIPTION

Overview of Apparatus

The present technology relates to a system and related methods for amplifying, and carrying out diagnostic analyses on, polynucleotides (e.g., a DNA, RNA, mRNA, or rRNA) from biological samples. For example, the system and methods can determine whether a polynucleotide indicative of the presence of a particular pathogen (such as a bacterium or a virus) can be present. The polynucleotide may be a sample of genomic DNA, or may be a sample of mitochondrial DNA. The nucleotides are typically provided to the system having been isolated or released from particles such as cells in the sample. The system includes a disposable microfluidic cartridge containing multiple sample lanes in parallel and a reusable instrument platform (a PCR analyzer apparatus) that can actuate on-cartridge operations, can detect (e.g., by fluorescence detection) and analyze the products of the PCR amplification in each of the lanes separately, in all simulta-20 neously, or in groups simultaneously, and, optionally, can display the results on a graphical user interface.

A system, microfluidic cartridge, heater unit, detector, kit, methods, and associated computer program product, are now further described.

By cartridge is meant a unit that may be disposable, or reusable in whole or in part, and that is configured to be used in conjunction with some other apparatus that has been suitably and complementarily configured to receive and operate on (such as deliver energy to) the cartridge.

By microfluidic, as used herein, is meant that volumes of sample, and/or reagent, and/or amplified polynucleotide are from about 0.1 µl to about 999 µl, such as from 1-100 µl, or from 2-25 µl. Similarly, as applied to a cartridge, the term microfluidic means that various components and channels of 35 the cartridge, as further described herein, are configured to accept, and/or retain, and/or facilitate passage of microfluidic volumes of sample, reagent, or amplified polynucleotide.

FIG. 1 shows a perspective view of an exemplary apparatus 100 consistent with those described herein, as well as various components thereof, such as exemplary cartridge 200 that contains multiple sample lanes, and exemplary read head 300 that contains detection apparatus for reading signals from cartridge 200. The apparatus 100 of FIG. 1 is able to carry out real-time PCR on a number of samples in cartridge 200 simultaneously. Preferably the number of samples is 12 samples, as illustrated with exemplary cartridge 200, though other numbers of samples such as 4, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 are within the scope of the present description. In preferred operation of the apparatus, a PCR-ready solution FIGS. 38-39 show positioning of a cartridge in an exem- 50 containing the sample, and, optionally, one or more analytespecific reagents (ASR's) is prepared, as further described elsewhere (see, e.g., U.S. patent application publication 2006-0166233, incorporated herein by reference), prior to introduction into cartridge 200. An exemplary kit for preparing a PCR-ready sample, for use with the system described herein, the kit comprising buffers, lysis pellets, and affinity pellets, is shown in FIG. 2.

System Overview

A schematic overview of a system 981 for carrying out analyses described herein is shown in FIG. 3. The geometric arrangement of the components of system 981 shown in FIG. 3, as well as their respective connectivities, is exemplary and not intended to be limiting.

A processor 980, such as a microprocessor, is configured to 65 control functions of various components of the system as shown, and is thereby in communication with each such component. In particular, processor 980 is configured to receive

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data about a sample to be analyzed, e.g., from a sample reader **990**, which may be a barcode reader, an optical character reader, or an RFID scanner (radio frequency tag reader). For example, the sample identifier can be a handheld bar code reader. Processor **980** can be configured to accept user 5 instructions from an input **984**, where such instructions may include instructions to start analyzing the sample, and choices of operating conditions.

Processor 980 can also be configured to communicate with an optional display 982, so that, for example, information 10 including but not limited to the current status of the system, progress of PCR thermocycling, and any warning message in case of malfunction of either system or cartridge, as well as results of analysis, are transmitted to the display. Additionally, processor 980 may transmit one or more questions to be 15 displayed on display 982 that prompt a user to provide input in response thereto. Thus, in certain embodiments, input 984 and display 982 are integrated with one another.

Processor 980 can be optionally further configured to transmit results of an analysis to an output device such as a 20 printer, a visual display, or a speaker, or a combination thereof, the transmission being either directly through a directly dedicated printer cable, or wirelessly, or via a network connection.

Processor 980 is still further optionally connected via a 25 communication interface such as a network interface to a computer network 988. The communication interface can be one or more interfaces selected from the group consisting of: a serial connection, a parallel connection, a wireless network connection and a wired network connection such as an ethernet, firewire, cable connection, or one using USB connectivity. Thereby, when the system is suitably addressed on the network, a remote user may access the processor and transmit instructions, input data, or retrieve data, such as may be stored in a memory (not shown) associated with the processor, or on 35 some other computer-readable medium that is in communication with the processor. The computer network connection may also permit extraction of data to a remote location, such as a personal computer, personal digital assistant, or network storage device such as computer server or disk farm. The 40 apparatus may further be configured to permit a user to e-mail results of an analysis directly to some other party, such as a healthcare provider, or a diagnostic facility, or a patient.

Although not shown in FIG. 3, in various embodiments, input 984 can include one or more input devices selected from 45 the group consisting of: a keyboard, a touch-sensitive surface, a microphone, a track-pad, and a mouse. A suitable input device may further comprise a reader of formatted electronic media, such as, but not limited to, a flash memory card, memory stick, USB-stick, CD, or floppy diskette. An input 50 device may further comprise a security feature such as a fingerprint reader, retinal scanner, magnetic strip reader, or bar-code reader, for ensuring that a user of the system is in fact authorized to do so, according to, for example, pre-loaded identifying characteristics of authorized users. An input 55 device may additionally—and simultaneously—function as an output device for writing data in connection with sample analysis. For example, if an input device is a reader of formatted electronic media, it may also be a writer of such media. Data that may be written to such media by such a 60 device includes, but is not limited to, environmental information, such as temperature or humidity, pertaining to an analysis, as well as a diagnostic result, and identifying data for the sample in question.

Additionally, in various embodiments, the apparatus can 65 further comprise a data storage medium configured to receive data from one or more of the processor, an input device, and

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a communication interface, the data storage medium being one or more media selected from the group consisting of: a hard disk drive, an optical disk drive, or one or more removable storage media such as a CD-R, CD-RW, USB-drive, and a flash card.

Processor 980 is further configured to control various aspects of sample diagnosis, as follows in overview, and as further described in detail herein. The system is configured to operate in conjunction with a complementary cartridge 994, such as a microfluidic cartridge. The cartridge is itself configured, as further described herein, to receive one or more samples 996 containing one or more polynucleotides in a form suitable for amplification and diagnostic analysis. The cartridge has dedicated regions within which amplification, such as by PCR, of the polynucleotides is carried out when the cartridge is situated in the apparatus.

The microfluidic cartridge is received by a receiving bay 992 configured to selectively receive the cartridge. For example, the receiving bay and the microfluidic cartridge can be complementary in shape so that the microfluidic cartridge is selectively received in, e.g., a single orientation. The microfluidic cartridge can have a registration member that fits into a complementary feature of the receiving bay. The registration member can be, for example, a cut-out on an edge of the cartridge, such as a corner that is cut-off, or one or more notches that are made on one or more of the sides. By selectively receiving the cartridge, the receiving bay can help a user to place the cartridge so that the apparatus can properly operate on the cartridge. The receiving bay can also be configured so that various components of the apparatus that can operate on the microfluidic cartridge (heat sources, detectors, force members, and the like) are positioned to properly operate on the microfluidic cartridge. In some embodiments, the apparatus can further include a sensor coupled to the processor, the sensor configured to sense whether the microfluidic cartridge is selectively received.

The receiving bay is in communication with a heater unit 998 that itself is controlled by processor 980 in such a way that specific regions of the cartridge, such as individual sample lanes, are independently and selectively heated at specific times during amplification and analysis. The processor can be configured to control application of heat to the individual sample lanes, separately, in all simultaneously, or in groups simultaneously.

The heat source can be, for example, a contact heat source such as a resistive heater or a network of resistive heaters, or a Peltier device, and the like. The contact heat source can be configured to be in direct physical contact with one or more distinct locations of a microfluidic cartridge received in the receiving bay. In various embodiments, each contact source heater can be configured to heat a distinct location having an average diameter in 2 dimensions from about 1 millimeter (mm) to about 15 mm (typically about 1 mm to about 10 mm), or a distinct location having a surface area of between about 1 mm² about 225 mm² (typically between about 1 mm² and about 100 mm², or in some embodiments between about 5 mm² and about 50 mm²).

In various embodiments, the heat source can be situated in an assembly that is removable from the apparatus, for example, to permit cleaning or to replace the heater configuration.

In various embodiments, the apparatus can include a compliant layer at the contact heat source configured to thermally couple the contact heat source with at least a portion of a microfluidic cartridge received in the receiving bay. The com-

pliant layer can have a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100

In various embodiments, the apparatus can further include one or more force members (not shown in FIG. 3) configured 5 to apply force to thermally couple the at least one heat source at least a portion of a microfluidic cartridge received in the receiving bay.

In various embodiments, the one or more force members are configured to apply force to a plurality of locations in the microfluidic cartridge. The force applied by the one or more force members can result in an average pressure at an interface between a portion of the receiving bay and a portion of the microfluidic cartridge of between about 5 kilopascals and about 50 kilopascals, for example, the average pressure can be at least about 7 kilopascals, and still more preferably at least about 14 kilopascals. At least one force member can be manually operated. At least one force member can be mechanically coupled to a lid at the receiving bay, whereby 20 operation of the lid operates the force member. The application of force is important to ensure consistent thermal contact between the heater wafer and the PCR reactor and microvalves in the microfluidic cartridge.

In various embodiments, the apparatus can further include 25 a lid at the receiving bay, the lid being operable to at least partially exclude ambient light from the receiving bay. The lid can be, for example, a sliding lid. The lid can include the optical detector. A major face of the lid at the optical detector or at the receiving bay can vary from planarity by less than 30 about 100 micrometers, for example, less than about 25 micrometers. The lid can be configured to be removable from the apparatus. The lid can include a latching member that ensures that the lid is securely closed before amplification reactions are applied to the samples in the cartridge.

The processor is also configured to receive signals from and control a detector 999 configured to detect a polynucleotide in a sample in one or more of the individual sample lanes, separately or simultaneously. The processor thereby provides an indication of a diagnosis from the cartridge 994. 40 Diagnosis can be predicated on the presence or absence of a specific polynucleotide in a particular sample. The diagnosis can be transmitted to the output device 986 and/or the display 982, as described hereinabove.

The detector can be, for example, an optical detector that 45 includes a light source that selectively emits light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. Alternatively, for 50 example, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof; or for example, the optical detector can be configured to independently detect a plurality of fluorescent 60 dyes at a plurality of different locations on a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof in a different sample.

A suitable processor 980 can be designed and manufac- 65 tured according to, respectively, design principles and semiconductor processing methods known in the art.

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The system in FIG. 3 is configured so that a cartridge with capacity to receive multiple samples can be acted upon by the system to analyze multiple samples—or subsets thereofsimultaneously, or to analyze the samples consecutively. It is also consistent that additional samples can be added to a cartridge, while previously added samples are being amplified and analyzed.

The system shown in outline in FIG. 3, as with other exemplary embodiments described herein, is advantageous at least because it does not require locations within the system suitably configured for storage of reagents. Neither does the system, or other exemplary embodiments herein, require inlet or outlet ports that are configured to receive reagents from, e.g., externally stored containers such as bottles, canisters, or reservoirs. Therefore, the system in FIG. 3 is self-contained and operates in conjunction with a microfluidic cartridge, wherein the cartridge has locations within it configured to receive mixtures of sample and PCR reagents.

The system of FIG. 3 may be configured to carry out operation in a single location, such as a laboratory setting, or may be portable so that it can accompany, e.g., a physician, or other healthcare professional, who may visit patients at different locations. The system is typically provided with a power-cord so that it can accept AC power from a mains supply or generator. An optional transformer (not shown) built into the system, or situated externally between a power socket and the system, transforms AC input power into a DC output for use by the system. The system may also be configured to operate by using one or more batteries and therefore is also typically equipped with a battery recharging system, and various warning devices that alert a user if battery power is becoming too low to reliably initiate or complete a diagnostic analysis.

The system of FIG. 3 may further be configured, for mul-35 tiplexed cartridge analysis. In one such configuration, multiple instances of a system, as outlined in FIG. 3, are operated in conjunction with one another to accept and to process multiple cartridges, where each cartridge has been loaded with a different sample. Each component shown in FIG. 3 may therefore be present as many times as there are cartridges, though the various components may be configured in a common housing.

In still another configuration, a system is configured to accept and to process multiple cartridges, but one or more components in FIG. 3 is common to multiple cartridges. For example, a single device may be configured with multiple cartridge receiving bays, but a common processor and user interface suitably configured to permit concurrent, consecutive, or simultaneous, control of the various cartridges. In such an embodiment a single detector, for example, can scan across all of the multiple cartridges. It is further possible that such an embodiment, also utilizes a single sample reader, and a single output device.

In still another configuration, a system as shown in FIG. 3 dye; or for example, the optical detector can be configured to 55 is configured to accept a single cartridge, but wherein the single cartridge is configured to process more than 1, for example, 2, 3, 4, 5, or 6, samples in parallel, and independently of one another.

It is further consistent with the present technology that a cartridge can be tagged, e.g., with a molecular bar-code indicative of one or more of the samples, to facilitate sample tracking, and to minimize risk of sample mix-up. Methods for such tagging are described elsewhere, e.g., in U.S. patent application publication Ser. No. 10/360,854, incorporated herein by reference.

In various embodiments, the apparatus can further include an analysis port. The analysis port can be configured to allow

an external sample analyzer to analyze a sample in the microfluidic cartridge; for example, the analysis port can be a hole or window in the apparatus which can accept an optical detection probe that can analyze a sample in situ in the microfluidic cartridge. In some embodiments, the analysis port can be 5 configured to direct a sample from the microfluidic cartridge to an external sample analyzer; for example, the analysis port can include a conduit in fluid communication with the microfluidic cartridge that direct a liquid sample to a chromatography apparatus, an optical spectrometer, a mass spectrometer, 10

Apparatus 100 may optionally comprise one or more stabilizing feet that cause the body of the device to be elevated above a surface on which system 100 is disposed, thereby permitting ventilation underneath system 100, and also pro- 15 viding a user with an improved ability to lift system 100. There may be 2, 3, 4, 5, or 6, or more feet, depending upon the size of system 100. Such feet are preferably made of rubber, or plastic, or metal, and in some embodiments may elevate the body of system 100 by from about 2 to about 10 mm above a 20 surface on which it is situated. The stabilizing function can also be provided by one or more runners that run along one or more edges—or are inwardly displaced from one or more edges—of the underside of the apparatus. Such runners can also be used in conjunction with one or more feet. In another 25 embodiment, a turntable situated on the underside permits the apparatus to be rotated in a horizontal or near-horizontal plane when positioned on, e.g., a benchtop, to facilitate access from a number of angles by a user.

FIG. 4 shows a schematic cross-sectional view of a part of an apparatus as described herein, showing input of sample into a cartridge 200 via a pipette 10 (such as a disposable pipette) and an inlet 202. Inlet 202 is preferably configured to receive a pipette or the bottom end of a PCR tube and thereby accept sample for analysis with minimum waste, and with minimum introduction of air. Cartridge 200 is disposed on top of and in contact with a heater substrate 400. Read head 300 is positioned above cartridge 200 and a cover for optics 310 restricts the amount of ambient light that can be detected by the read head.

FIG. 5 shows an example of 4-pipette head used for attaching disposable pipette tips, prior to dispensing PCR-ready sample into a cartridge.

**Exemplary Systems** 

or the like.

FIGS. **6A-6**E show exterior perspective views of various 45 configurations of an exemplary system, as further described herein. FIG. **6A** shows a perspective view of a system **2000** for receiving microfluidic cartridge (not shown), and for causing and controlling various processing operations to be performed a sample introduced into the cartridge. The elements 50 of system **2000** are not limited to those explicitly shown. For example, although not shown, system **2000** may be connected to a hand-held bar-code reader, as further described herein.

System 2000 comprises a housing 2002, which can-be made of metal, or a hardened plastic. The form of the housing 55 shown in FIG. 6A embodies stylistic as well as functional features. Other embodiments of the technology may appear somewhat differently, in their arrangement of the components, as well as their overall appearance, in terms of smoothness of lines, and of exterior finish, and texture. System 2000 further comprises one or more stabilizing members 2004. Shown in FIG. 6A is a stabilizing foot, of which several are normally present, located at various regions of the underside of system 2000 so as to provide balance and support. For example, there may be three, four, five, six, or eight such 65 stabilizing feet. The feet may be moulded into and made of the same material as housing 2002, or may be made of one or

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more separate materials and attached to the underside of system 2000. For example, the feet may comprise a rubber that makes it hard for system 2000 to slip on a surface on which it is situated, and also protects the surface from scratches. The stabilizing member of members may take other forms than feet, for example, rails, runners, or one or more pads.

System 2000 further comprises a display 2006, which may be a liquid crystal display, such as active matrix, an OLED, or some other suitable form. It may present images and other information in color or in black and white. Display 2006 may also be a touch-sensitive display and therefore may be configured to accept input from a user in response to various displayed prompts. Display 2006 may have an anti-reflective coating on it to reduce glare and reflections from overhead lights in an laboratory setting. Display 2006 may also be illuminated from, e.g., a back-light, to facilitate easier viewing in a dark laboratory.

System 2000, as shown in FIG. 6A, also comprises a moveable lid 2010, having a handle 2008. The lid 2010 can slide back and forward. In FIG. 6A, the lid is in a forward position, whereby it is "closed". In FIG. 6B, the lid is shown in a back position, wherein the lid is "open" and reveals a receiving bay 2014 that is configured to receive a microfluidic cartridge. Of course, as one of ordinary skill in the art would appreciate, the technology described herein is not limited to a lid that slides, or one that slides back and forward. Side to side movement is also possible, as is a configuration where the lid is "open" when positioned forward in the device. It is also possible that the lid is a hinged lid, or one that is totally removable.

Handle 2008 performs a role of permitting a user to move lid 2010 from one position to another, and also performs a role of causing pressure to be forced down on the lid, when in a closed position, so that pressure can be applied to a cartridge in the receiving bay 2014. In FIG. 6C, handle 2008 is shown in a depressed position, wherein force is thereby applied to lid 2014, and thus pressure is applied to a cartridge received in the receiving bay beneath the lid.

In one embodiment, the handle and lid assembly are also fitted with a mechanical sensor that does not permit the handle to be depressed when there is no cartridge in the receiving bay. In another embodiment, the handle and lid assembly are fitted with a mechanical latch that does not permit the handle to be raised when an analysis is in progress.

A further configuration of system 2000 is shown in FIG. 6D, wherein a door 2012 is in an open position. Door 2012 is shown in a closed position in FIGS. 6A-C. The door is an optional component that permits a user to access a heater module 2020, and also a computer-readable medium input tray 2022. System 2000 can function without a door that covers heater module 2020 and medium input 2022, but such a door has convenience attached to it. Although the door 2012 is shown hinged at the bottom, it may also be hinged at one of its sides, or at its upper edge. Door 2012 may alternatively be a removable cover, instead of being hinged. Door 2012, may also be situated at the rear, or side of system 2000 for example, if access to the heater module and/or computer readable medium input is desired on a different face of the system. It is also consistent with the system herein that the heater module, and the computer readable medium input are accessed by separate doors on the same or different sides of the device, and wherein such separate doors may be independently hinged or removable.

Heater module **2020** is preferably removable, and is further described hereinbelow.

Computer readable medium input **2022** may accept one or more of a variety of media. Shown in FIG. **2D** is an exemplary

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form of input 2022, a CD-Rom tray for accepting a CD, DVD, or mini-CD, or mini-DVD, in any of the commonly used readable, read-writable, and writable formats. Also consistent with the description herein is an input that can accept another form of medium, such as a floppy disc, flash memory such as memory stick, compact flash, smart data-card, or secure-data card, a pen-drive, portable USB-drive, zip-disk, and others. Such an input can also be configured to accept several different forms of media. Such an input 2022 is in communication with a processor (as described in connection with FIG. 3, though not shown in FIGS. 6A-E), that can read data from a computer-readable medium when properly inserted into the input.

FIG. 6E shows a plan view of a rear of system 2000. Shown are an air vent 2024, or letting surplus heat escape during an analysis. Typically, on the inside of system 2000, and by air vent 2024 and not shown in FIG. 6E, is a fan. Other ports shown in FIG. 6E are as follows: a power socket 2026 for accepting a power cord that will connect system 2000 to a 20 supply of electricity; an ethernet connection 2028 for linking system 2000 to a computer network such as a local area network; an phone jack connection 2032 for linking system 2000 to a communication network such as a telephone network; one or more USB ports 2030, for connecting system 25 2000 to one or more peripheral devices such as a printer, or a computer hard drive; an infra-red port for communicating with, e.g., a remote controller (not shown), to permit a user to control the system without using a touch-screen interface. For example, a user could remotely issue scheduling commands 30 to system 2000 to cause it to start an analysis at a specific time in the future.

Features shown on the rear of system 2000 may be arranged in any different manner, depending upon an internal configuration of various components. Additionally, features 35 shown as being on the rear of system 2000, may be optionally presented on another face of system 2000, depending on design preference. Shown in FIG. 6E are exemplary connections. It would be understood that various other features, including inputs, outputs, sockets, and connections, may be 40 present on the rear face of system 2000, though not shown, or on other faces of system 2000.

An exploded view of an exemplary embodiment of the apparatus is shown in FIG. 7, particularly showing internal features of apparatus 2000. Apparatus 2000 can comprise a 45 computer readable medium configured with hardware/firmware that can be employed to drive and monitor the operations on a cartridge used therewith, as well as software to interpret, communicate and store the results of a diagnostic test performed on a sample processed in the cartridge. Referring to 50 FIG. 7, typical components of the apparatus 2000 are shown and include, for example, control electronics 2005, removable heater/sensor module 2020, detector 2009 such as a fluorescent detection module, display screen or optionally combined display and user interface 2006 (e.g., a medical 55 grade touch sensitive liquid crystal display (LCD)). In some embodiments, lid 2010, detector 2009, and handle 2008 can be collectively referred to as slider module 2007. Additional components of apparatus 2000 may include one or more mechanical fixtures such as frame 2019 to hold the various 60 modules (e.g., the heater/sensor module 2020, and/or the slider module 2007) in alignment, and for providing structural rigidity. Detector module 2009 can be placed in rails to facilitate opening and placement of cartridge 2060 in the apparatus 2000, and to facilitate alignment of the optics upon 65 closing. Heater/sensor module 2020 can be also placed on rails for easy removal and insertion of the assembly.

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Embodiments of apparatus 2000 also include software (e.g., for interfacing with users, conducting analysis and/or analyzing test results), firmware (e.g., for controlling the hardware during tests on the cartridge 812), and one or more peripheral communication interfaces shown collectively as 2031 for peripherals (e.g., communication ports such as USB/Serial/Ethernet to connect to storage such as compact disc or hard disk, to connect input devices such as a bar code reader and/or a keyboard, to connect to other computers or storage via a network, and the like).

Control electronics 840, shown schematically in the block diagram in FIG. 8, can include one or more functions in various embodiments, for example for, main control 900, multiplexing 902, display control 904, detector control 906, and the like. The main control function may serve as the hub of control electronics 840 in apparatus 2000 and can manage communication and control of the various electronic functions. The main control function can also support electrical and communications interface 908 with a user or an output device such as a printer 920, as well as optional diagnostic and safety functions. In conjunction with main control function 900, multiplexer function 902 can control sensor data 914 and output current 916 to help control heater/sensor module 2020. The display control function 904 can control output to and, if applicable, interpret input from touch screen LCD 846, which can thereby provide a graphical interface to the user in certain embodiments. The detector function 906 can be implemented in control electronics 840 using typical control and processing circuitry to collect, digitize, filter, and/or transmit the data from a detector 2009 such as one or more fluorescence detection modules.

#### Microfluidic Cartridge

The present technology comprises a microfluidic cartridge that is configured to carry out an amplification, such as by PCR, of one or more polynucleotides from one or more samples. It is to be understood that, unless specifically made clear to the contrary, where the term PCR is used herein, any variant of PCR including but not limited to real-time and quantitative, and any other form of polynucleotide amplification is intended to be encompassed. The microfluidic cartridge need not be self-contained and can be designed so that it receives thermal energy from one or more heating elements present in an external apparatus with which the cartridge is in thermal communication. An exemplary such apparatus is further described herein; additional embodiments of such a system are found in U.S. patent application Ser. No. 11/940,310, entitled "Microfluidic Cartridge and Method of Making Same", and filed on even date herewith, the specification of which is incorporated herein by reference.

By cartridge is meant a unit that may be disposable, or reusable in whole or in part, and that is configured to be used in conjunction with some other apparatus that has been suitably and complementarily configured to receive and operate on (such as deliver energy to) the cartridge.

By microfluidic, as used herein, is meant that volumes of sample, and/or reagent, and/or amplified polynucleotide are from about 0.1  $\mu l$  to about 999  $\mu l$ , such as from 1-100  $\mu l$ , or from 2-25  $\mu l$ . Similarly, as applied to a cartridge, the term microfluidic means that various components and channels of the cartridge, as further described herein, are configured to accept, and/or retain, and/or facilitate passage of microfluidic volumes of sample, reagent, or amplified polynucleotide. Certain embodiments herein can also function with nanoliter volumes (in the range of 10-500 nanoliters, such as 100 nanoliters).

One aspect of the present technology relates to a microfluidic cartridge having two or more sample lanes arranged so

that analyses can be carried out in two or more of the lanes in parallel, for example simultaneously, and wherein each lane is independently associated with a given sample.

A sample lane is an independently controllable set of elements by which a sample can be analyzed, according to 5 methods described herein as well as others known in the art. A sample lane comprises at least a sample inlet, and a microfluidic network having one or more microfluidic components, as further described herein.

In various embodiments, a sample lane can include a sample inlet port or valve, and a microfluidic network that comprises, in fluidic communication one or more components selected from the group consisting of: at least one thermally actuated valve, a bubble removal vent, at least one thermally actuated pump, a gate, mixing channel, positioning 15 element, microreactor, a downstream thermally actuated valve, and a PCR reaction chamber. The sample inlet valve can be configured to accept a sample at a pressure differential compared to ambient pressure of between about 70 and 100 kilopascals.

The cartridge can therefore include a plurality of microfluidic networks, each network having various components, and each network configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined.

A multi-lane cartridge is configured to accept a number of samples in series or in parallel, simultaneously or consecutively, in particular embodiments 12 samples, wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain 30 one or more polynucleotides in a form suitable for amplification. The polynucleotides in question may be the same as, or different from one another, in different samples and hence in different lanes of the cartridge. The cartridge typically processes each sample by increasing the concentration of a polynucleotide to be determined and/or by reducing the concentration of inhibitors relative to the concentration of polynucleotide to be determined.

The multi-lane cartridge comprises at least a first sample lane having a first microfluidic network and a second lane 40 having a second microfluidic network, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network is configured to amplify polynucleotides in the first sample, and wherein the second microfluidic 45 network is configured to amplify polynucleotides in the second sample.

In various embodiments, the microfluidic network can be configured to couple heat from an external heat source to a sample mixture comprising PCR reagent and neutralized 50 polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

At least the external heat source may operate under control of a computer processor, configured to execute computer 55 readable instructions for operating one or more components of each sample lane, independently of one another, and for receiving signals from a detector that measures fluorescence from one or more of the PCR reaction chambers.

For example, FIG. 9 shows a plan view of a microfluidic 60 cartridge 100 containing twelve independent sample lanes 101 capable of simultaneous or successive processing. The microfluidic network in each lane is typically configured to carry out amplification, such as by PCR, on a PCR-ready sample, such as one containing nucleic acid extracted from a 65 sample using other methods as further described herein. A PCR-ready sample is thus typically a mixture comprising the

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PCR reagents and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide sample. For example, a PCR-ready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence. Exemplary probes are further described herein. Typically, the microfluidic network is configured to couple heat from an external heat source with the mixture comprising the PCR reagent and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

In various embodiments, the PCR reagent mixture can include a positive control plasmid and a plasmid fluorogenic hybridization probe selective for at least a portion of the plasmid, and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe.

In various embodiments, the microfluidic cartridge can <sup>25</sup> accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide. Each lane of a multi-lane cartridge as described herein can perform two reactions when used in conjunction with two fluorescence detection systems per lane. A variety of combinations of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two other lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

FIG. 10A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. FIG. 10B shows a close-up view of a portion of the cartridge 200 of FIG. 10A illustrating various representative components. The cartridge 200 may be referred to as a multi-lane PCR cartridge with dedicated sample inlets 202. For example sample inlet 202 is configured to accept a liquid transfer member (not shown) such as a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown in FIGS. 10A, 10B, wherein one inlet operates in conjunction with a single sample lane. Various components of microfluidic circuitry in each lane are also visible. For example, microvalves 204, and 206, and hydrophobic vents 208 for removing air bubbles, are parts of microfluidic circuitry in a given lane. Also shown is an ultrafast PCR reactor 210, which, as further described herein, is a microfluidic channel in a given sample lane that is long enough to permit PCR to amplify polynucleotides present in a sample. Above each PCR reactor 210 is a window 212 that permits detection of fluorescence from a fluorescent substance in PCR reactor 210 when a detector is situated above window 212. It is to be understood that other configurations of windows are possible including, but not limited to, a single window that straddles each PCR reactor across the width of cartridge 200.

In preferred embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is

15 customarily used in the art. Advantageously, then, such a cartridge may be used with plate handlers used elsewhere in

The sample inlets of adjacent lanes are reasonably spaced apart from one another to prevent any contamination of one 5 sample inlet from another sample when a user introduces a sample into any one cartridge. In an embodiment, the sample inlets are configured so as to prevent subsequent inadvertent introduction of sample into a given lane after a sample has already been introduced into that lane. In certain embodiments, the multi-sample cartridge is designed so that a spacing between the centroids of sample inlets is 9 mm, which is an industry-recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge that accept samples from PCR tubes, as further described herein, is 9 mm. The inlet holes can be manufactured conical in shape with an appropriate conical angle so that industry-standard pipette tips  $(2 \mu l, 20 \mu l, 200 \mu l,$ volumes, etc.) fit snugly therein. The cartridge herein may be adapted to suit other, later-arising, industry standards not 20 layer 428, 430 of polypropylene or other plastic label with otherwise described herein, as would be understood by one of ordinary skill in the art.

In one embodiment, an exemplary microfluidic cartridge has 12 sample lanes. The inlet ports in this embodiment have a 6 mm spacing, so that, when used in conjunction with an 25 automated sample loader having 4 heads, spaced equidistantly at 18 mm apart, the inlets can be loaded in three batches of four inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from one side of the cartridge to the other as shown.

A microfluidic cartridge as used herein may be constructed from a number of layers. Accordingly, one aspect of the present technology relates to a microfluidic cartridge that comprises a first, second, third, fourth, and fifth layers 35 wherein one or more layers define a plurality of microfluidic networks, each network having various components configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined. In various embodiments, one or more such layers are 40 optional.

FIGS. 11A-C show various views of a layer structure of an exemplary microfluidic cartridge comprising a number of layers, as further described herein. FIG. 11A shows an exploded view; FIG. 11B shows a perspective view; and FIG. 45 11C shows a cross-sectional view of a sample lane in the exemplary cartridge. Referring to FIGS. 11A-C, an exemplary microfluidic cartridge 400 includes first 420, second 422, third 424, fourth 426, and fifth layers in two non-contiguous parts 428, 430 (as shown) that enclose a microfluidic 50 network having various components configured to process multiple samples in parallel that include one or more polynucleotides to be determined.

Microfluidic cartridge 400 can be fabricated as desired. The cartridge can include a microfluidic substrate layer 424, 55 typically injection molded out of a plastic, such as a zeonor plastic (cyclic olefin polymer), having a PCR channel and valve channels on a first side and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a second side (disposed toward hydrophobic vent mem- 60 brane 426). It is advantageous that all the microfluidic network defining structures, such as PCR reactors, valves, inlet holes, and air vents, are defined on the same single substrate **424**. This attribute facilitates manufacture and assembly of the cartridge. Additionally, the material from which this sub- 65 strate is formed is rigid or nondeformable, non-venting to air and other gases, and has a low autofluorescence to facilitate

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detection of polynucleotides during an amplification reaction performed in the microfluidic circuitry defined therein. Rigidity is advantageous because it facilitates effective and uniform contact with a heat unit as further described herein. Use of a non-venting material is also advantageous because it reduces the likelihood that the concentration of various species in liquid form will change during analysis. Use of a material having low auto-fluorescence is also important so that background fluorescence does not detract from measurement of fluorescence from the analyte of interest.

The cartridge can further include, disposed on top of the substrate 424, an oleophobic/hydrophobic vent membrane layer 426 of a porous material, such as 0.2 to 1.0 micron pore-size membrane of modified polytetrafluorethylene, the membrane being typically between about 25 and about 100 microns thick, and configured to cover the vent channels of microfluidic substrate 424, and attached thereto using, for example, heat bonding.

Typically, the microfluidic cartridge further includes a pressure sensitive adhesive (typically between about 50 and 150 microns thick) configured to seal the wax loading holes of the valves in substrate 424, trap air used for valve actuation, and serve as a location for operator markings. In FIG. 4A, this layer is shown in two separate pieces, 428, 430, though it would be understood by one of ordinary skill in the art that a single piece layer would be appropriate.

In various embodiments, the label is a computer-readable label. For example, the label can include a bar code, a radio frequency tag or one or more computer-readable characters. The label can be foamed of a mechanically compliant material. For example, the mechanically compliant material of the label can have a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100. The label can be positioned such that it can be read by a sample identification verifier as further described herein.

The cartridge can further include a heat sealable laminate layer 422 (typically between about 100 and about 125 microns thick) attached to the bottom surface of the microfluidic substrate 424 using, for example, heat bonding. This layer serves to seal the PCR channels and vent channels in substrate 424. The cartridge can further include a thermal interface material layer 420 (typically about 125 microns thick), attached to the bottom of the heat sealable laminate layer using, for example, pressure sensitive adhesive. The layer 420 can be compressible and have a higher thermal conductivity than common plastics, thereby serving to transfer heat across the laminate more efficiently. Typically, however, layer 420 is not present.

The application of pressure to contact the cartridge to the heater of an instrument that receives the cartridge generally assists in achieving better thermal contact between the heater and the heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was only partially filled with liquid and the air entrapped therein would be thermally expanded during thermocycling.

In use, cartridge 400 is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, actuators, and processing region 410) of the device. Exemplary such heater arrays are further described herein. Additional embodiments of heater arrays are described in U.S. patent application Ser. No. 11/940,315, entitled "Heater Unit for Microfluidic Diagnostic System" and filed on even date herewith, the specification of which is incorporated herein by reference in its entirety. In some embodiments, the heat sources are controlled by a computer

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processor and actuated according to a desired protocol. Processors configured to operate microfluidic devices are described in, e.g., U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference.

In various embodiments, during transport and storage, the microfluidic cartridge can be further surrounded by a sealed pouch. The microfluidic cartridge can be sealed in the pouch with an inert gas. The microfluidic cartridge can be disposable for example after one or more of its sample lanes have 10 been used.

Highly Multiplexed Embodiments

Embodiments of the cartridge described herein may be constructed that have high-density microfluidic circuitry on a single cartridge that thereby permit processing of multiple 15 samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 20, 24, 36, 40, 48, 50, 60, 64, 72, 80, 84, 96, and 100, but it would be understood that still other numbers are consistent with the apparatus and cartridge herein, where deemed convenient and 20 practical

Accordingly, different configurations of lanes, sample inlets, and associated heater networks than those explicitly depicted in the FIGs and examples that can facilitate processing such numbers of samples on a single cartridge are within 25 the scope of the instant disclosure. Similarly, alternative configurations of detectors and heating elements for use in conjunction with such a highly multiplexed cartridge are also within the scope of the description herein.

It is also to be understood that the microfluidic cartridges 30 described herein are not to be limited to rectangular shapes, but can include cartridges having circular, elliptical, triangular, rhombohedral, square, and other shapes. Such shapes may also be adapted to include some irregularity, such as a cut-out, to facilitate placement in a complementary apparatus as further described herein.

In an exemplary embodiment, a highly multiplexed cartridge has 48 sample lanes, and permits independent control of each valve in each lane by suitably configured heater circuitry, with 2 banks of thermo cycling protocols per lane, as 40 shown in FIG. 12. In the embodiment in FIG. 12, the heaters (shown superimposed on the lanes) are arranged in three arrays 502, 504, with 506, and 508. The heaters are themselves disposed within one or more substrates. Heater arrays **502**, **508** in two separate glass regions only apply heat to 45 valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and passed to the PCR reaction chambers independently of one 50 another. The PCR heaters 504,506 are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. It is preferable for the PCR 55 heaters to be arranged in 2 banks (the heater arrays 506 on the left and right 508 are not in electrical communication with one another), thereby permitting a separate degree of sample

FIG. 13 shows a representative 48-sample cartridge 600 compatible with the heater arrays of FIG. 12, and having a configuration of inlets 602 different to that depicted in other cartridges herein. The inlet configuration is exemplary and has been designed to maximize efficiency of space usage on the cartridge. The inlet configuration can be compatible with 65 an automatic pipetting machine that has dispensing heads situated at a 9 mm spacing. For example, such a machine

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having 4 heads can load 4 inlets at once, in 12 discrete steps, for the cartridge of FIG. 13. Other configurations of inlets though not explicitly described or depicted are compatible with the technology described herein.

FIG. 14 shows, in close up, an exemplary spacing of valves 702, channels 704, and vents 796, in adjacent lanes 708 of a multi-sample microfluidic cartridge for example as shown in FIG. 13.

FIGS. 15 and 16 show close-ups of, respectively, heater arrays 804 compatible with, and inlets 902 on, the exemplary cartridge shown in FIG. 14.

FIGS. 17A and 17B show various views of an embodiment of a radially-configured highly-multiplexed cartridge, having a number of inlets 1002, microfluidic lanes 1004, valves 1005, and PCR reaction chambers 1006. FIG. 17C shows an array of heater elements 1008 compatible with the cartridge layout of FIG. 17A.

The various embodiments shown in FIGS. 12-17C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the other specific examples described herein.

During the design and manufacture of highly multiplexed cartridges, photolithographic processing steps such as etching, hole drilling/photo-chemical drilling/sand-blasting/ion-milling processes should be optimized to give well defined holes and microchannel pattern. Proper distances between channels should be identified and maintained to obtain good bonding between the microchannel substrate and the heat conducting substrate layer. In particular, it is desirable that minimal distances are maintained between pairs of adjacent microchannels to promote, reliable bonding of the laminate in between the channels.

The fabrication by injection molding of these complicated microfluidic structures having multiple channels and multiple inlet holes entails proper consideration of dimensional repeatability of these structures over multiple shots from the injection molding master pattern. Proper consideration is also attached to the placement of ejector pins to push out the structure from the mold without causing warp, bend or stretching of it. For example, impression of the ejector pins on the microfluidic substrate should not sink into the substrate thereby preventing planarity of the surface of the cartridge. The accurate placement of various inlet holes (such as sample inlet holes, valve inlet holes and vent holes) relative to adjacent microfluidic channels is also important because the presence of these holes can cause knit-lines to form that might cause unintended leak from a hole to a microchannel. Highly multiplexed microfluidic substrates may be fabricated in other materials such as glass, silicon.

The size of the substrate relative to the number of holes is also factor during fabrication because it is easy to make a substrate having just a simple microfluidic network with a few holes (maybe fewer than 10 holes) and a few microchannels, but making a substrate having over 24, or over 48, or over 72 holes, etc., is more difficult.

Microfluidic Networks

Particular components of exemplary microfluidic networks are further described herein.

Channels of a microfluidic network in a lane of cartridge typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

FIG. 18 shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. 10A and 10B. It would be understood by one

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skilled in the art that other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In operation of the cartridge, in sequence, sample is introduced through liquid inlet 202, optionally flows into a bubble removal vent channel 208 (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel 216. Typically, when using a robotic dispenser of liquid sample, the volume is dispensed accurately enough that formation of bubbles is not a significant problem, and the presence of vent channel 208 is not necessary. Thus, in certain embodiments, the bubble removal vent channel 208 is not present and sample flows directly into channel 216. Throughout the operation of cartridge 200, the fluid is manipulated as a microdroplet (not shown in the FIGs). Valves 204 and 206 are initially both open, so that a microdroplet of samplecontaining fluid can be pumped into PCR reactor channel 210 from inlet hole 202 under influence of force from the sample injection operation. Upon initiating of processing, the detec- 20 tor present on top of the PCR reactor 210 checks for the presence of liquid in the PCR channel, and then valves 204 and 206 are closed to isolate the PCR reaction mix from the outside. In one embodiment, the checking of the presence of liquid in the PCR channel is by measuring the heat ramp rate, 25 such as by one or more temperature sensors in the heating unit. A channel with liquid absent will heat up faster than one in which, e.g., a sample, is present.

Both valves 204 and 206 are closed prior to thermocycling to prevent or reduce any evaporation of liquid, bubble gen- 30 eration, or movement of fluid from the PCR reactor. End vent 214 is configured to prevent a user from introducing an excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample 35 Valves volumes as small as an amount to fill the region from the bubble removal vent (if present) to the middle of the microreactor, or up to valve 204 or beyond valve 204. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully com- 40 plete a PCR thermocycling reaction.

The reactor 210 is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein, and according to amplification protocols known to those of ordinary skill 45 in the art. The inside walls of the channel in the PCR reactor are typically made very smooth and polished to a shiny finish (for example, using a polish selected from SPI A1, SPI A2, SPI A3, SPI B1, or SPI B2) during manufacture. This is in order to minimize any microscopic quantities of air trapped in 50 the surface of the PCR channel, which would causing bubbling during the thermocycling steps. The presence of bubbles especially in the detection region of the PCR channel could also cause a false or inaccurate reading while monitoring progress of the PCR. Additionally, the PCR channel can 55 be made shallow such that the temperature gradient across the depth of the channel is minimized.

The region of the cartridge 212 above PCR reactor 210 is a thinned down section to reduce thermal mass and autofluorescence from plastic in the cartridge. It permits a detector to 60 more reliably monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. Exemplary probes are further described herein. The region 212 can be made of thinner material than the rest of the cartridge so as to permit the PCR channel to be 65 more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denatur20

ing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence.

After PCR has been carried out on a sample, and presence or absence of a polynucleotide of interest has been determined, it is preferred that the amplified sample remains in the cartridge and that the cartridge is either used again (if one or more lanes remain unused), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the laminate of the cartridge, and recover an amount—typically about 1.5 microliter—of PCR product. The user may also place the individual PCR lane on a special narrow heated plate, maintained at a temperature to melt the wax in the valve, and then aspirate the reacted sample from the inlet hole of that PCR

In various embodiments, the microfluidic network can optionally include at least one reservoir configured to contain

Table 1 outlines typical volumes, pumping pressures, and operation times associated with various components of a microfluidic cartridge described herein.

TABLE 1

Operation	Pumping Pressure	Displacement Volume	Time of Operation
Moving valve wax plugs	~1-2 psi	<1 µl	5-15 seconds
Operation	Pump Used	Pump Design	Pump Actuation
Moving valve wax plugs	Thermopneumatic pump	1 μl of trapped air	Heat trapped air to ~70-90 C.

A valve (sometimes referred to herein as a microvalve) is a component in communication with a channel, such that the valve has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Upon actuation of the valve, the valve transitions to a closed state that prevents material from passing along the channel from one side of the valve to the other. For example, in one embodiment, a valve can include a mass of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage when the valve is closed. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The TRS can also be a blend of variety of materials, such as an emulsion of thermoelastic polymer blended with air microbubbles (to enable higher thermal expansion, as well as reversible expansion and contraction), polymer blended with expancel material (offering higher thermal expansion), polymer blended with heat conducting microspheres (offering faster heat conduction and hence, faster melting profiles), or a polymer blended with magnetic microspheres (to permit magnetic actuation of the melted thermoresponsive material).

Generally, for such a valve, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less). Typically, a chamber is in gaseous communication with the mass of TRS.

The valve is in communication with a source of heat that can be selectively applied to the chamber of air and to the TRS. Upon heating gas (e.g., air) in the chamber and heating the mass of TRS to the second temperature, gas pressure within the chamber due to expansion of the volume of gas, forces the mass to move into the channel, thereby obstructing material from passing therealong.

An exemplary valve is shown in FIG. 19A. The valve of FIG. 19A has two chambers of air 1203, 1205 in contact with, respectively, each of two channels 1207, 1208 containing TRS. The air chambers also serve as loading ports for TRS during manufacture of the valve, as further described herein. In order to make the valve sealing very robust and reliable, the flow channel 1201 (along which, e.g., sample passes) at the valve junction is made narrow (typically 150 μm wide, and 150 µm deep or narrower), and the constricted portion of the flow channel is made at least 0.5 or 1 mm long such that the TRS seals up a long narrow channel thereby reducing any leakage through the walls of the channel. In the case of a bad seal, there may be leakage of fluid around walls of channel, 20 past the TRS, when the valve is in the closed state. In order to minimize this, the flow channel is narrowed and elongated as much as possible. In order to accommodate such a length of channel on a cartridge where space may be at a premium, the flow channel can incorporate one or more curves 1209 as 25 shown in FIG. 19A. The valve operates by heating air in the TRS-loading port, which forces the TRS forwards into the flow-channel in a manner so that it does not come back to its original position. In this way, both air and TRS are heated during operation.

In various other embodiments, a valve for use with a microfluidic network in a microfluidic cartridge herein can be a bent valve as shown in FIG. 19B. Such a configuration reduces the footprint of the valve and hence reduces cost per part for highly dense microfluidic cartridges. A single valve 35 loading hole 1211 is positioned in the center, that serves as an inlet for thermally responsive substance. The leftmost vent 1213 can be configured to be an inlet for, e.g., sample, and the rightmost vent 1215 acts as an exit for, e.g., air. This configuration can be used as a prototype for testing such attributes as 40 valve and channel geometry and materials.

In various other embodiments, a valve for use with a microfluidic network can include a curved valve as shown in FIG. 19C, in order to reduce the effective cross-section of the valve, thereby enabling manufacture of cheaper dense 45 microfluidic devices. Such a valve can function with a single valve loading hole and air chamber 1221 instead of a pair as shown in FIG. 19A.

Gates

FIG. 19D shows an exemplary gate as may optionally be 50 used in a microfluidic network herein. A gate can be a component that can have a closed state that does not allow material to pass along a channel from a position on one side of the gate to another side of the gate, and an open state that does allow material to pass along a channel from a position on one 55 side of the gate to another side of the gate. Actuation of an open gate can transition the gate to a closed state in which material is not permitted to pass from one side of the gate (e.g., upstream of the gate). Upon actuation, a closed gate can transition to an open state in which material is permitted to pass from one side of the gate (e.g., upstream of the gate) to the other side of the gate (e.g., downstream of the gate) to the other side of the gate (e.g., downstream of the gate).

In various embodiments, a microfluidic network can include a narrow gate **380** as shown in FIG. **19**D where a gate 65 loading channel **382** used for loading wax from a wax loading hole **384** to a gate junction **386** can be narrower (e.g., approxi-

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mately 150 µm wide and 100 microns deep). An upstream channel 388 as well as a downstream channel 390 of the gate junction 386 can be made wide (e.g., ~500 μm) and deep (e.g., ~500 µm) to help ensure the wax stops at the gate junction 386. The amount of gate material melted and moved out of the gate junction 386 may be minimized for optimal gate 380 opening. As an off-cartridge heater may be used to melt the thermally responsive substance in gate 380, a misalignment of the heater could cause the wax in the gate loading channel **382** to be melted as well. Therefore, narrowing the dimension of the loading channel may increase reliability of gate opening. In the case of excessive amounts of wax melted at the gate junction 386 and gate loading channel 382, the increased cross-sectional area of the downstream channel 390 adjacent to the gate junction 386 can prevent wax from clogging the downstream channel 390 during gate 380 opening. The dimensions of the upstream channel 388 at the gate junction 386 can be made similar to the downstream channel 390 to ensure correct wax loading during gate fabrication.

In various embodiments, the gate can be configured to minimize the effective area or footprint of the gate within the network and thus bent gate configurations, although not shown herein are consistent with the foregoing description.

In various embodiments, the microfluidic network can include at least one hydrophobic vent in addition to an end vent. A vent is a general outlet (hole) that may or may not be covered with a hydrophobic membrane. An exit hole is an example of a vent that need not be covered by a membrane.

A hydrophobic vent (e.g., a vent in FIG. 20) is a structure that permits gas to exit a channel while limiting (e.g., preventing) quantities of liquid from exiting the channel. Typically, hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from GE Osmonics, Minnetonka, Minn.) that defines a wall of the channel. As described elsewhere herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microfluidic network.

The hydrophobic vents of the present technology are preferably constructed so that the amount of air that escapes through them is maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane 1303 of large surface area and a shallow cross section of the microchannel below the vent surface.

Hydrophobic vents are useful for bubble removal and typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel **1305** (see FIG. **13**). The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less).

The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of the depth of the channel upstream 1301 and downstream (not shown) of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns. Other dimensions are consistent with the description herein.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and

downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns, and the width of the channel upstream and downstream from the vent is about 250 microns. Other dimensions are consistent with the description herein.

The vent in FIG. 20 is shown in a linear configuration though it would be understood that it need not be so. A bent, kinked, curved, S-shaped, V-shaped, or U-shaped (as in item 208 FIG. 11) vent is also consistent with the manner of 10 construction and operation described herein.

Use of Cutaways in Cartridge and Substrate To Improve Rate of Cooling During PCR Cycling

During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved effi- 15 ciency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the heating substrate and/or the cartridge, as shown in FIG. 21.

One way to achieve rapid cooling is to cutaway portions of 20 the microfluidic cartridge substrate, as shown in FIG. 22A. The upper panel of FIG. 22A is a cross-section of an exemplary microfluidic cartridge taken along the dashed line A-A' as marked on the lower panel of FIG. 22A. PCR reaction chamber 1601, and representative heaters 1603 are shown. 25 Also shown are two cutaway portions, one of which labeled **1601**, that are situated alongside the heaters that are positioned along the long side of the PCR reaction chamber. Cutaway portions such as 1601 reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction chamber. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology.

Another way to achieve rapid cooling is to cutaway portions of the heater substrate, as shown in FIG. 22B. The lower panel of FIG. 22B is a cross-section of an exemplary microfluidic cartridge and heater substrate taken along the dashed line A-A' as marked on the upper panel of FIG. 22B. PCR 40 reaction chamber 901, and representative heaters 1003 are shown. Also shown are four cutaway portions, one of which labeled 1205, that are situated alongside the heaters that are situated along the long side of the PCR reaction chamber. Cutaway portions such as 1205 reduce the thermal mass of the 45 heater substrate, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction chamber. Four separate cutaway portions are shown in FIG. 22A so that control circuitry to the various 50 heaters is not disrupted. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology. These cutouts may be created by a method selected from: selective etching using wet etching processes, deep reactive ion etching, selective etching using 55 heat bonded to, the top of the microfluidic substrate covering CO2 laser or femtosecond laser (to prevent surface cracks or stress near the surface), selective mechanical drilling, selective ultrasonic drilling, or selective abrasive particle blasting. Care has to be taken to maintain mechanically integrity of the heater while reducing as much material as possible.

FIG. 22C shows a combination of cutouts and use of ambient air cooling to increase the cooling rate during the cooling stage of thermocycling. A substantial amount of cooling happens by convective loss from the bottom surface of the heater surface to ambient air. The driving force for this convective 65 loss is the differential in temperatures between the glass surface and the air temperature. By decreasing the ambient air

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temperature by use of, for example, a peltier cooler, the rate of cooling can be increased. The convective heat loss may also be increased by keeping the air at a velocity higher than zero.

An example of thermal cycling performance in a PCR reaction chamber obtained with a configuration as described herein, is shown in FIG. 23 for a protocol that is set to heat up the reaction mixture to 92° C., and maintain the temperature for 1 second, then cool to 62° C., and stay for 10 seconds. The cycle time shown is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C. To minimize the overall time required for a PCR effective to produce detectable quantities of amplified material, it is important to minimize the time required for each cycle. Cycle times in the range 15-30 s, such as 18-25 s, and 20-22 s, are desirable. In general, an average PCR cycle time of 25 seconds as well as cycle times as low as 20 seconds are typical with the technology described herein. Using reaction volumes less than a microliter (such as a few hundred nanoliters or less) permits use of an associated smaller PCR chamber. and enables cycle times as low as 15 seconds. An average cycle time of 25 seconds and as low as 20 seconds can be achieved by technology described herein, even without any forced cooling or implementing any thermal mass reductions described elsewhere herein.

Manufacturing Process for Cartridge

FIG. 24 shows a flow-chart 1800 for an embodiment of an assembly process for an exemplary cartridge as shown in FIG. 11A herein. It would be understood by one of ordinary skill in the art, both that various steps may be performed in a different order from the order set forth in FIG. 24, and additionally that any given step may be carried out by alternative methods to those described in the figure. It would also be understood that, where separate serial steps are illustrated for carrying out two 35 or more functions, such functions may be performed synchronously and combined into single steps and remain consistent with the overall process described herein.

At 1802, a laminate layer is applied to a microfluidic substrate that has previously been engineered, for example by injection molding, to have a microfluidic network constructed in it; edges are trimmed from the laminate where they spill over the bounds of the substrate.

At 1804, wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate. An exemplary process for carrying this out is further described herein.

At 1806, the substrate is inspected to ensure that wax from step 1804 is loaded properly and that the laminate from step **1802** adheres properly to it. If a substrate does not satisfy either or both of these tests, it is usually discarded. If substrates repeatedly fail either or both of these tests, then the wax dispensing, or laminate application steps, as applicable,

At 1808, a hydrophobic vent membrane is applied to, and at least the one or more vent holes, and on the opposite face of the substrate from the laminate. Edges of the membrane that are in excess of the boundary of the substrate are trimmed.

At 1810, the assembly is inspected to ensure that the hydro-60 phobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is discarded, and, in the case of repeated discard events, the foregoing process step 1808 is reviewed.

At 1812, optionally, a thermally conductive pad layer is applied to the bottom laminate of the cartridge.

At **1814**, two label strips are applied to the top of the microfluidic substrate, one to cover the valves, and a second to protect the vent membranes. It would be understood that a single label strip may be devised to fulfill both of these roles.

At **1816**, additional labels are printed or applied to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Preferably one or more of these labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

Optionally, at **1818**, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked, and cartridges packed into groups, such as groups of 25, or groups of 10, or groups of 20, or groups of 48 or 50. Preferably the packaging is via an inert and/or moisture-free medium. Wax Loading in Valves

In general, a valve as shown in, e.g., FIGS. 25A-C, is constructed by depositing a precisely controlled amount of a TRS (such as wax) into a loading inlet machined in the microfluidic substrate. FIGS. 25A and 25B show how a combination of controlled hot drop dispensing into a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The top of FIG. 25A shows a plan view of a valve inlet 190 and loading channel 1902, 25 connecting to a flow channel 1904. The lower portions of FIG. 25A show the progression of a dispensed wax droplet 1906 (having a volume of 75 nl±15 nl) through the inlet 1901 and into the loading channel 1902.

To accomplish those steps, a heated dispenser head can be accurately positioned over the inlet hole of the micro channel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of 20%. A suitable dispenser is also one that can deposit amounts smaller than 100 nl with a precision of +/-20%. The dispenser should also be capable of heating and maintaining the dispensing temperature of the TRS to be dispensed. For example, it may have a reservoir to hold the solution of TRS. It is also desirable that the dispense head can have freedom of movement at least in a horizontal (x-y) plane so that it can easily move to various locations of a microfluidic substrate and dispense volumes of TRS into valve inlets at such locations without having to be re-set, repositioned manually, or recalibrated in between each dispense operation.

The inlet hole of the microfluidic cartridge, or other microchannel device, is dimensioned in such a way that the droplet of 75 nl can be accurately propelled to the bottom of the inlet hole using, for example, compressed air, or in a manner similar to an inkjet printing method. The microfluidic cartridge is maintained at a temperature above the melting point of the wax thereby permitting the wax to stay in a molten state immediately after it is dispensed. After the drop falls to the bottom of the inlet hole 1901, the molten wax is drawn into the narrow channel by capillary action, as shown in the sequence of views in FIG. 25B. A shoulder between the inlet hole 1901 and the loading channel can facilitate motion of the TRS. The volume of the narrow section can be designed to be approximately equal to a maximum typical amount that is dispensed into the inlet hole. The narrow section can also be designed so that even though the wax dispensed may vary considerably between a minimum and a maximum shot size, the wax always fills up to, and stops at, the micro channel junction 1907 because the T-junction provides a higher cross 65 section than that of the narrow section and thus reduces the capillary forces.

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PCR Reagent Mixtures

In various embodiments, the sample for introduction into a lane of the microfluidic cartridge can include a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides.

In various embodiments, preparation of a PCR-ready sample for use with an apparatus and cartridge as described herein can include contacting a neutralized polynucleotide sample with a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid).

The PCR-ready sample can be prepared, for example, using the following steps: (1) collect sample in sample collection buffer, (2) transfer entire sample to lysis tube, mix, heat, and incubate for seven minutes, (3) place on magnetic rack, allow beads to separate, aspirate supernatant, (4) add 100 μl of Buffer 1, mix, place on magnetic rack, allow beads to separate, aspirate supernatant, (5) add 10 μl of Buffer 2, mix, place in high temperature heat block for 3 minutes, place on magnetic rack, allow beads to separate, transfer 5 μl supernatant, and (6) Add 5 μl of Buffer 3, transfer 1 to 10 μl of supernatant for PCR amplification and detection.

The PCR reagent mixture can be in the form of one or more lyophilized pellets and the steps by which the PCR-ready sample is prepared can involve reconstituting the PCR pellet by contacting it with liquid to create a PCR reagent mixture solution. In yet another embodiment, each of the PCR lanes may have dried down or lyophilized ASR reagents preloaded such that the user only needs to input prepared polynucleotide sample into the PCR. In another embodiment, the PCR lanes may have only the application-specific probes and primers pre-measured and pre-loaded, and the user inputs a sample mixed with the PCR reagents.

In various embodiments, the PCR-ready sample can include at least one probe that can be selective for a polynucleotide sequence, wherein the steps by which the PCR-ready sample is prepared involve contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the probe. The probe can be a fluorogenic hybridization probe. The fluorogenic hybridization probe can include a polynucleotide sequence coupled to a fluorescent reporter dye and a fluorescence quencher dye.

In various embodiments, the PCR-ready sample further includes a sample buffer.

In various embodiments, the PCR-ready sample includes at least one probe that is selective for a polynucleotide sequence, e.g., the polynucleotide sequence that is characteristic of a pathogen selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the PCR reagent mixture can further include a polymerase enzyme, a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism, for example any organism that employs deoxyribonucleic acid or ribonucleic acid polynucleotides. Thus, the probe can be selective for any organism. Suitable organisms include mammals (including humans), birds, reptiles, amphibians, fish, domesticated animals, wild animals, extinct organisms, bacteria, fungi, viruses, plants, and the like. The probe can also be selective for components of organisms that employ their own polynucleotides, for example mitochondria. In some embodiments, the probe is selective for microorgan-

isms, for example, organisms used in food production (for example, yeasts employed in fermented products, molds or bacteria employed in cheeses, and the like) or pathogens (e.g., of humans, domesticated or wild mammals, domesticated or wild mammals, domesticated or

of humans, domesticated or wild mammals, domesticated or wild birds, and the like). In some embodiments, the probe is 5 selective for organisms selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi,

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protozoa, and viruses.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism 10 selected from the group consisting of Staphylococcus spp., e.g., S. epidermidis, S. aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Staphylococcus; Streptococcus (e.g.,  $\alpha$ ,  $\beta$  or  $\gamma$ -hemolytic, Group A, B, C, D or G) such as S. pyogenes, S. agalactiae; E. faecalis, E. 15 durans, and E. faecium (formerly S. faecalis, S. durans, S. faecium); nonenterococcal group D streptococci, e.g., S. bovis and S. equines; Streptococci viridans, e.g., S. mutans, S. sanguis, S. salivarius, S. mitior, A. milleri, S. constellatus, S. intermedius, and S. anginosus; S. iniae; S. pneumoniae; Neis- 20 seria, e.g., N. meningitides, N gonorrhoeae, saprophytic Neisseria sp; Erysipelothrix, e.g., E. rhusiopathiae; Listeria spp., e.g., L. monocytogenes, rarely L. ivanovii and L. seeligeri; Bacillus, e.g., B. anthracis, B. cereus, B. subtilis, B. subtilis subtilus niger, B. thuringiensis; Nocardia asteroids; 25 Legionella, e.g., L. pneumophila, Pneumocystis, e.g., P. carinii; Enterobacteriaceae such as Salmonella, Shigella, Escherichia (e.g., E. coli, E. coli O157:H7); Klebsiella, Enterobacter, Serratia, Proteus, Morganella, Providencia, Yersinia, and the like, e.g., Salmonella, e.g., S. typhi S. 30 paratyphi A, B (S. schottmuelleri), and C (S. hirschfeldii), S. dublin S. choleraesuis, S. enteritidis, S. typhimurium, S. heidelberg, S. newport, S. infantis, S. agona, S. montevideo, and S. saint-paul; Shigella e.g., subgroups: A, B, C, and D, such as S. flexneri, S. sonnei, S. boydii, S. dysenteriae; Pro- 35 teus (P. mirabilis, P. vulgaris, and P. myxofaciens), Morganella (M. morganii); Providencia (P. rettgeri, P. alcalifaciens, and P. stuartii); Yersinia, e.g., Y. pestis, Y. enterocolitica; Haemophilus, e.g., H. influenzae, H. parainfluenzae, H. aphrophilus, H. ducreyi; Brucella, e.g., B. abor- 40 tus, B. melitensis, B. suis, B. canis; Francisella, e.g., F. tularensis; Pseudomonas, e.g., P. aeruginosa, P. paucimobilis, P. putida, P. fluorescens, P. acidovorans, Burkholderia (Pseudomonas) pseudomallei, Burkholderia Burkholderia cepacia and Stenotrophomonas maltophilia; 45 Campylobacter, e.g., C. fetus fetus, C. jejuni, C. pylori (Helicobacter pylori); Vibrio, e.g., V. cholerae, V. parahaemolyticus, V. mimicus, V. alginolyticus, V. hollisae, V. vulnificus, and the nonagglutinable vibrios; Clostridia, e.g., C. perfringens, C. tetani, C. difficile, C. botulinum; Actinomyces, e.g., A. 50 israelii; Bacteroides, e.g., B. fragilis, B. thetaiotaomicron, B. distasonis, B. vulgatus, B. ovatus, B. caccae, and B. merdae; Prevotella, e.g., P. melaminogenica; genus Fusobacterium; Treponema, e.g. T. pallidum subspecies endemicum, T. pallidum subspecies pertenue, T. carateum, and T. pallidum sub- 55 species pallidum; genus Borrelia, e.g., B burgdorferi; genus Leptospira; Streptobacillus, e.g., S. moniliformis; Spirillum, e.g., S. minus; Mycobacterium, e.g., M. tuberculosis, M. bovis, M. africanum, M. avium M. intracellulare, M. kansasii, M. xenopi, M. marinum, M. ulcerans, the M. fortuitum com- 60 plex (M. fortuitum and M. chelonea, M. leprae, M. asiaticum, M. chelonea subsp. abscessus, M. fallax, M. fortuitum, M. malmoense, M. shimoidei, M. simiae, M. szulgai, M. xenopi; Mycoplasma, e.g., M. hominis, M. orale, M. salivarium, M. fermentans, M. pneumoniae, M. bovis, M. tuberculosis, M. 65 avium, M. leprae; Mycoplasma, e.g., M. genitalium; Ureaplasma, e.g., U. urealyticum; Trichomonas, e.g., T. vaginalis;

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Cryptococcus, e.g., C. neoformans; Histoplasma, e.g., H. capsulatum; Candida, e.g., C. albicans; Aspergillus sp; Coccidioides, e.g., C. immitis; Blastomyces, e.g. B. dermatitidis; Paracoccidioides, e.g., P. brasiliensis; Penicillium, e.g., P. marneffei; Sporothrix, e.g., S. schenckii; Rhizopus, Rhizomucor, Absidia, and Basidiobolus; diseases caused by Bipolaris. Cladophialophora, Cladosporium, Drechslera, Exophiala, Fonsecaea, Phialophora, Xylohypha, Ochroconis, Rhinocladiella, Scolecobasidium, and Wangiella; Trichosporon, e.g., T. beigelii; Blastoschizomyces, e.g., B. capitatus; Plasmodium, e.g., P. falciparum, P. vivax, P. ovale, and P. malariae; Babesia sp; protozoa of the genus Trypanosoma, e.g., T. cruzi; Leishmania, e.g., L. donovani, L. major L. tropica, L. mexicana, L. braziliensis, L. viannia braziliensis; Toxoplasma, e.g., T. gondii; Amoebas of the genera Naegleria or Acanthamoeba; Entamoeba histolytica; Giardia lamblia; genus Cryptosporidium, e.g., C. parvum; Isospora belli; Cyclospora cayetanensis; Ascaris lumbricoides; Trichuris trichiura; Ancylostoma duodenale or Necator americanus; Strongyloides stercoralis Toxocara, e.g., T. canis, T. cati; Baylisascaris, e.g., B. procyonis; Trichinella, e.g., T. spiralis; Dracunculus, e.g., D. medinensis; genus Filarioidea; Wuchereria bancrofti; Brugia, e.g., B. malayi, or B. timori; Onchocerca volvulus; Loa loa; Dirofilaria immitis; genus Schistosoma, e.g., S. japonicum, S. mansoni, S. mekongi, S. intercalatum, S. haematobium; Paragonimus, e.g., P. Westermani, P. Skrjabini; Clonorchis sinensis; Fasciola hepatica; Opisthorchis sp; Fasciolopsis bush; Diphyllobothrium latum; Taenia, e.g., T. saginata, T. solium; Echinococcus, e.g., E. granulosus, E. multilocularis; Picornaviruses, rhinoviruses echoviruses, coxsackieviruses, influenza virus; paramyxoviruses, e.g., types 1, 2, 3, and 4; adenoviruses; Herpesviruses, e.g., HSV-1 and HSV-2; varicella-zoster virus; human T-lymphotropic virus (type I and type II); Arboviruses and Arenaviruses; Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae; Flavivirus; Hantavirus; Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]); Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo]); Smallpox (variola); retroviruses e.g., human immunodeficiency viruses 1 and 2; human papillomavirus [HPV] types 6, 11, 16, 18, 31, 33, and 35.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxvtoca, Klebsiella pneumoniae, Escherichia coli, Acinetobacter Baumannii, Serratia marcescens, Enterobacter aerogenes, Enterococcus faecium, vancomycin-resistant enterococcus (VRE), Staphylococcus aureus. methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus viridans, Listeria monocytogenes, Enterococcus spp., Streptococcus Group B, Streptococcus Group C, Streptococcus Group G, Streptococcus Group F, Enterococcus faecalis, Streptococcus pneumoniae, Staphylococcus epidermidis, Gardnerella vaginalis, Micrococcus sps., Haemophilus influenzae, Neisseria gonorrhoeae, Moraxella catarrhalis, Salmonella sps., Chlamydia trachomatis, Peptostreptococcus productus, Peptostreptococcus anaerobius, Lactobacillus fermentum, Eubacterium lentum, Candida glabrata, Candida albicans, Chlamydia spp., Campylobacter Camplobacter spp., Salmonella spp., smallpox (variola major), Yersinia Pestis, Herpes Simplex Virus I (HSV I), and Herpes Simplex Virus II (HSV II).

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of Group B *Streptococcus*.

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In various embodiments, a method of carrying out PCR on a sample can further include one or more of the following steps: heating the biological sample in the microfluidic cartridge; pressurizing the biological sample in the microfluidic cartridge at a pressure differential compared to ambient pressure of between about 20 kilopascals and 200 kilopascals, or in some embodiments, between about 70 kilopascals and 110 kilopascals.

In some embodiments, the method for sampling a polynucleotide can include the steps of: placing a microfluidic cartridge containing a PCR-ready sample in a receiving bay of a suitably configured apparatus; carrying out PCR on the sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide in the sample, the PCR-ready sample comprising a polymerase 15 enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid, and a plurality of nucleotides; contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the at least one fluorogenic probe that is selective for a polynucleotide 20 sequence, wherein the probe is selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses; and detecting the fluorogenic probe, the presence of the organism for which the 25 one fluorogenic probe is selective is determined.

Carrying out PCR on a PCR-ready sample can additionally include: independently contacting each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; and/or contacting the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon 35 thereof with at least one probe that is selective for a polynucleotide sequence.

In various embodiments, a method of using the apparatus and cartridge described herein can further include one or more of the following steps: determining the presence of a 40 polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; determining that the sample was contaminated if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof; and/or in some embodiments, wherein the PCR reagent mixture further comprises a positive control plasmid and a plasmid probe selective for at least a portion of the plasmid, the method further including determining that a PCR amplification has occurred 50 if the plasmid probe is detected.

In various embodiments, the microfluidic cartridge as described herein can be provided in the form of a kit, wherein the kit can include a microfluidic cartridge, and a liquid 55 transfer member (such as a syringe or a pipette). In various embodiments, the kit can further include instructions to employ the liquid transfer member to transfer a sample containing extracted nucleic acid from a sample container via a sample inlet to the microfluidic network on the microfluidic cartridge. In some embodiments, the microfluidic cartridge and the liquid transfer member can be sealed in a pouch with an inert gas.

Typically when transferring a sample from liquid dispenser, such as a pipette tip, to an inlet on the microfluidic 65 cartridge, a volume of air is simultaneously introduced into the microfluidic network, the volume of air being between

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about 0.5 mL and about 5 mL. Presence of air in the microfluidic network, however, is not essential to operation of the cartridge described herein.

In various embodiments, the kit can further include at least one computer-readable label on the cartridge. The label can include, for example, a bar code, a radio frequency tag or one or more computer-readable characters. When used in conjunction with a similar computer-readable label on a sample container, such as a vial or a pouch, matching of diagnostic results with sample is thereby facilitated.

In some embodiments, a sample identifier of the apparatus described elsewhere herein is employed to read a label on the microfluidic cartridge and/or a label on the biological sample. Heater Unit

An exemplary heater unit 2020 is shown in FIG. 26. The unit is configured to deliver localized heat to various selected regions of a cartridge received in a receiving bay 2014. Heater unit 2020 is configured to be disposed within a diagnostic apparatus during operation, as further described herein, and in certain embodiments is removable from that apparatus, for example to facilitate cleaning, or to permit reconfiguration of the heater circuitry. In various embodiments, heater unit 2020 can be specific to particular designs of microfluidic networks and microfluidic substrate layouts.

Shown in FIG. 26 is a heater unit having a recessed surface 2044 that provides a platform for supporting a microfluidic cartridge when in receiving bay 2014. In one embodiment, the cartridge rests directly on surface 2044. Surface 2044 is shown as recessed, in FIG. 2, but need not be so and, for example, may be raised or may be flush with the surrounding area of the heater unit. Surface 2044 is typically a layer of material that overlies a heater chip or board, or a heater substrate, that contains heater micro-circuitry configured to selectively and specifically heat regions of a microfluidic substrate, such as in a cartridge, in the receiving bay 2014.

Area 2044 is configured to accept a microfluidic cartridge in a single orientation. Therefore area 2044 can be equipped with a registration member such as a mechanical key that prevents a user from placing a cartridge into receiving bay 2014 in the wrong configuration. Shown in FIG. 26 as an exemplary mechanical key 2045 is a diagonally cutout corner of area 2044 into which a complementarily cutoff corner of a microfluidic cartridge fits. Other registration members are consistent with the heater unit described herein, for example, a feature engineered on one or more edges of a cartridge including but not limited to: several, such as two or more, cut-out corners, one or more notches cut into one or more edges of the cartridge; or one or more protrusions fabricated into one or more edges of the cartridge. Alternative registration members include one or more lugs or bumps engineered into an underside of a cartridge, complementary to one or more recessed sockets or holes in surface 2044 (not shown in the embodiment of FIG. 26). Alternative registration members include one or more recessed sockets or holes engineered into an underside of a cartridge, complementary to one or more lugs or bumps on surface 2044. In general, the pattern of features is such that the cartridge possesses at least one element of asymmetry so that it can only be inserted in a single orientation into the receiving bay.

Also shown in FIG. 26 is a hand-grasp 2042 that facilitates removal and insertion of the heater unit into an apparatus by a user. Cutaway 2048 permits a user to easily remove a cartridge from receiving bay 2014 after a processing run where, e.g., a user's thumb or finger when grabbing the top of the cartridge, is afforded comfort space by cutaway 2048. Both cutaways 2042 and 2048 are shown as semicircular recesses in the embodiment of FIG. 26, but it would be understood that

they are not so limited in shape. Thus, rectangular, square, triangular, half-oval, contoured, and other shaped recesses are

also consistent with a heater unit as described herein.

In the embodiment of FIG. 26, which is designed to be compatible with an exemplary apparatus as further described 5 herein, the front of the heater unit is at the left of the figure. At the rear of heater unit 2020 is an electrical connection 2050, such as an RS-232 connection, that permits electrical signals to be directed to heaters located at specific regions of area 2044 during sample processing and analysis, as further 10 described herein. Thus, underneath area 2044 and not shown in FIG. 2 can be an array of heat sources, such as resistive heaters, that are configured to align with specified locations of a microfluidic cartridge properly inserted into the receiving bay. Surface 2044 is able to be cleaned periodically, for 15 example with common cleaning agents (e.g., a 10% bleach solution), to ensure that any liquid spills that may occur during sample handling do not cause any short circuiting. Such cleaning can be carried out frequently when the heater unit is disposed in a diagnostic apparatus, and less frequently 20 but more thoroughly when the unit is removed.

Other non-essential features of heater unit 2020 are as follows. One or more air vents 2052 can be situated on one or more sides (such as front, rear, or flanking) or faces (such as top or bottom) of heater unit 2020, to permit excess heat to 25 escape, when heaters underneath receiving bay 2014, are in operation. The configuration of air vents in FIG. 26, as a linear array of square vents, is exemplary and it would be understood that other numbers and shapes thereof are consistent with routine fabrication and use of a heater unit. For example, 30 although 5 square air vents are shown, other numbers such as 1, 2, 3, 4, 6, 8, or air vents are possible, arranged on one side, or spread over two or more sides and/or faces of the heater unit. In further embodiments, air vents may be circular, rectangular, oval, triangular, polygonal, and having curved or 35 squared vertices, or still other shapes, including irregular shapes. In further embodiments two or more vents need not be disposed in a line, parallel with one another and with an edge of the heater unit but may be disposed offset from one another.

Heater unit 2020 may further comprise one or more guid- 40 ing members 2047 that facilitate inserting the heater unit into an apparatus as further described herein, for an embodiment in which heater unit 2020 is removable by a user. Heater unit is advantageously removable because it permits system 2000 to be easily reconfigured for a different type of analysis, such 45 as employing a different cartridge with a different registration member and/or microfluidic network, in conjunction with the same or a different sequence of processing operations. In other embodiments, heater unit 2020 is designed to be fixed and only removable, e.g., for cleaning, replacement, or maintenance, by the manufacturer or an authorized maintenance agent, and not routinely by the user. Guiding members 2047 may perform one or more roles of ensuring that the heater unit is aligned correctly in the apparatus, and ensuring that the heater unit makes a tight fit and does not significantly move 55 during processing and analysis of a sample, or during transport of the apparatus.

Guiding members shown in the embodiment of FIG. 26 are on either side of receiving bay 2044 and stretch along a substantial fraction of the length of unit 2020, but such an 60 arrangement of guiding members is exemplary. Other guiding members are consistent with use herein, and include but are not limited to other numbers of guiding members such as 1, 3, 4, 5, 6, or 8, and other positions thereof, including positioned in area 2051 of unit 2020, and need not stretch along as much 65 of the length of unit 2020 as shown in FIG. 26, or may stretch along its entire length. Guiding members 2047 are shown

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having a non-constant thickness along their lengths. It is consistent herein that other guiding members may have essentially constant thickness along their lengths. At the end of the heater unit that is inserted into an apparatus, in the embodiment shown, the edges are beveled to facilitate proper placement.

Also shown in FIG. 26 is an optional region of fluorescent material, such as optically fluorescent material 2069, on area 2051 of heater unit 2020. The region of fluorescent material is configured to be detected by a detection system further described herein. The region 2069 is used for verifying the state of optics in the detection system prior to sample processing and analysis and therefore acts as a control, or a standard. For example, in one embodiment a lid of the apparatus in which the heater unit is disposed, when in an open position, permits ambient light to reach region 2069 and thereby cause the fluorescent material to emit a characteristic frequency or spectrum of light that can be measured by the detector for, e.g., standardization or calibration purposes. In another embodiment, instead of relying on ambient light to cause the fluorescent material to fluoresce, light source from the detection system itself, such as one or more LED's, is used to shine on region 2069. The region 2069 is therefore positioned to align with a position of a detector. Region 2069 is shown as rectangular, but may be configured in other shapes such as square, circular, elliptical, triangular, polygonal, and having curved or squared vertices. It is also to be understood that the region 2069 may be situated at other places on the heater unit 2020, according to convenience and in order to be complementary to the detection system deployed.

In particular and not shown in FIG. 26, heater/sensor unit 2020 can include, for example, a multiplexing function in a discrete multiplexing circuit board (MUX board), one or more heaters (e.g., a microheater), one or more temperature sensors (optionally combined together as a single heater/ sensor unit with one or more respective microheaters, e.g., as photolithographically fabricated on fused silica substrates). The micro-heaters can provide thermal energy that can actuate various microfluidic components on a suitably positioned microfluidic cartridge. A sensor (e.g., as a resistive temperature detector (RTD)) can enable real time monitoring of the micro-heaters, for example through a feedback based mechanism to allow for rapid and accurate control of the temperature. One or more microheaters can be aligned with corresponding microfluidic components (e.g., valves, pumps, gates, reaction chambers) to be heated on a suitably positioned microfluidic cartridge. A microheater can be designed to be slightly bigger than the corresponding microfluidic component(s) on the microfluidic cartridge so that even though the cartridge may be slightly misaligned, such as off-centered, from the heater, the individual components can be heated effectively.

Heater Configurations to Ensure Uniform Heating of a Region

The microfluidic substrates described herein are configured to accept heat from a contact heat source, such as found in a heater unit described herein. The heater unit typically comprises a heater board or heater chip that is configured to deliver heat to specific regions of the microfluidic substrate, including but not limited to one or more microfluidic components, at specific times. For example, the heat source is configured so that particular heating elements are situated adjacent to specific components of the microfluidic network on the substrate. In certain embodiments, the apparatus uniformly controls the heating of a region of a microfluidic network. In an exemplary embodiment, multiple heaters can be configured to simultaneously and uniformly heat a region,

such as the PCR reaction chamber, of the microfluidic substrate. The term heater unit, as used herein, may be used interchangeably to describe either the heater board or an item such as shown in FIG. 26.

Referring to FIGS. 27A and 27B, an exemplary set of 5 heaters configured to heat, cyclically, PCR reaction chamber 1001 is shown. It is to be understood that heater configurations to actuate other regions of a microfluidic cartridge such as other gates, valves, and actuators, may be designed and deployed according to similar principles to those governing 10 the heaters shown in FIGS. 27A and 27B.

Referring to FIGS. 27A and 27B, an exemplary PCR reaction chamber 1001 in a microfluidic substrate, typically a chamber or channel having a volume ~1.6 μl, is configured with a long side and a short side, each with an associated 15 heating element. A PCR reaction chamber may also be referred to as a PCR reactor, herein, and the region of a cartridge in which the reaction chamber is situated may be called a zone. The heater substrate therefore includes four heaters disposed along the sides of, and configured to heat, a 20 given PCR reaction chamber, as shown in the exemplary embodiment of FIG. 27A: long top heater 1005, long bottom heater 1003, short left heater 1007, and short right heater 1009. The small gap between long top heater 1005 and long bottom heater 1003 results in a negligible temperature gradi- 25 ent (less than 1° C. difference across the width of the PCR channel at any point along the length of the PCR reaction chamber) and therefore an effectively uniform temperature throughout the PCR reaction chamber. The heaters on the short edges of the PCR reactor provide heat to counteract the 30 gradient created by the two long heaters from the center of the reactor to the edge of the reactor.

It would be understood by one of ordinary skill in the art that still other configurations of one or more heater(s) situated about a PCR reaction chamber are consistent with the methods and apparatus described herein. For example, a 'long' side of the reaction chamber can be configured to be heated by two or more heaters. Specific orientations and configurations of heaters are used to create uniform zones of heating even on substrates having poor thermal conductivity because the poor 40 thermal conductivity of glass, or quartz, polyimide, FR4, ceramic, or fused silica substrates is utilized to help in the independent operation of various microfluidic components such as valves and independent operation of the various PCR lanes. It would be further understood by one of ordinary skill 45 in the art, that the principles underlying the configuration of heaters around a PCR reaction chamber are similarly applicable to the arrangement of heaters adjacent to other components of the microfluidic cartridge, such as actuators, valves, and gates.

Generally, the heating of microfluidic components, such as a PCR reaction chamber, is controlled by passing currents through suitably configured microfabricated heaters. Under control of suitable circuitry, the lanes of a multi-lane cartridge can then be controlled independently of one another. This can lead to a greater energy efficiency of the apparatus, because not all heaters are heating at the same time, and a given heater is receiving current for only that fraction of the time when it is required to heat. Control systems and methods of controllably heating various heating elements are further described in U.S. patent application Ser. No. 11/940,315, filed Nov. 14, 2007 and entitled "Heater Unit for Microfluidic Diagnostic System".

In certain embodiments, each heater has an associated temperature sensor. In the embodiment of FIG. 27A, a single 65 temperature sensor 1011 is used for both long heaters. A temperature sensor 1013 for short left heater, and a tempera-

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ture sensor 1015 for short right heater are also shown. The temperature sensor in the middle of the reactor is used to provide feedback and control the amount of power supplied to the two long heaters, whereas each of the short heaters has a dedicated temperature sensor placed adjacent to it in order to control it. As further described herein, temperature sensors are preferably configured to transmit information about temperature in their vicinity to a processor in the apparatus at such times as the heaters are not receiving current that causes them to heat. This can be achieved with appropriate control of current cycles.

In order to reduce the number of sensor or heater elements required to control a PCR heater, the heaters may be used to sense as well as heat, and thereby obviate the need to have a separate dedicated sensor for each heater. In another embodiment, each of the four heaters may be designed to have an appropriate wattage, and connect the four heaters in series or in parallel to reduce the number of electronically-controllable elements from four to just one, thereby reducing the burden on the associated electronic circuitry.

FIG. 27B shows expanded views of heaters and temperature sensors used in conjunction with a PCR reaction chamber of FIG. 27A. Temperature sensors 1001 and 1013 are designed to have a room temperature resistance of approximately 200-300 ohms. This value of resistance is determined by controlling the thickness of the metal layer deposited (e.g., a sandwich of 400 Å TiW/3,000 Å Au/400 Å TiW), and etching the winding metal line to have a width of approximately 10-25 μm and 20-40 mm length. The use of metal in this layer gives it a temperature coefficient of resistivity of the order of 0.5-20° C./ohms, preferably in the range of 1.5-3° C./ohms. Measuring the resistance at higher temperatures enables determination of the exact temperature of the location of these sensors.

The configuration for uniform heating, shown in FIG. 27A for a single PCR reaction chamber, can also be applied to a multi-lane PCR cartridge in which multiple independent PCR reactions occur.

Each heater can be independently controlled by a processor and/or control circuitry used in conjunction with the apparatus described herein. FIG. 27C shows thermal images, from the top surface of a microfluidic cartridge when heated by heaters configured as in FIGS. 27A and 27B, when each heater in turn is activated, as follows: (A): Long Top only; (B) Long Bottom only; (C) Short Left only; (D) Short Right only; and (E) All Four Heaters on. Panel (F) shows a view of the reaction chamber and heaters on the same scale as the other image panels in FIG. 27C. Also shown in the figure is a temperature bar.

The configuration for uniform heating, shown in FIG. 27A for a single PCR reaction chamber, can be applied to a multilane PCR cartridge in which multiple independent PCR reactions occur. See, e.g., FIG. 28, which shows an array of heater elements suitable to heat a cartridge herein.

Heater Multiplexing (Under Software Control)

Another aspect of the heater unit described herein, relates to a control of heat within the system and its components. The method leads to a greater energy efficiency of the apparatus described herein, because not all heaters are heating at the same time, and a given heater is receiving current for only part of the time.

Generally, the heating of microfluidic components, such as a PCR reaction chamber, is controlled by passing currents through suitably configured microfabricated heaters. The heating can be further controlled by periodically turning the current on and off with varying pulse width modulation (PWM), wherein pulse width modulation refers to the on-

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time/off-time ratio for the current. The current can be supplied by connecting a microfabricated heater to a high voltage source (for example, 30 V), which can be gated by the PWM signal. In some embodiments, the device includes 48 PWM signal generators. Operation of a PWM generator includes 5 generating a signal with a chosen, programmable, period (the end count) and a particular granularity. For instance, the signal can be 4000 µs (micro-seconds) with a granularity of 1 μs, in which case the PWM generator can maintain a counter beginning at zero and advancing in increments of 1 µs until it 10 reaches 4000 µs, when it returns to zero. Thus, the amount of heat produced can be adjusted by adjusting the end count. A high end count corresponds to a greater length of time during which the microfabricated heater receives current and therefore a greater amount of heat produced. It would be under- 15 stood that the granularity and signal width can take values other than those provided here without departing from the principles described herein.

Fluorescence Detection System, Including Lenses and Filters, and Multiple Parallel Detection for a Multi-Lane Cartridge

The detection system herein is configured to monitor fluorescence coming from one or more species involved in a biochemical reaction. The system can be, for example, an optical detector having a light source that selectively emits 25 light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof, as further described elsewhere herein. Alternatively, the optical 30 detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye. For example, the optical detector can be configured to independently detect a 35 plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. For example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality 40 of different locations of, for example, a microfluidic substrate, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. The detector further has potential for 2, 3 or 4 color detection and is controlled by software, preferably custom software, config- 45 ured to sample information from the detector.

The detection system described herein is capable of detecting a fluorescence signal from nanoliter scale PCR reactions. Advantageously, the detector is formed from inexpensive components, having no moving parts. The detector can be 50 configured to couple to a microfluidic cartridge as further described herein, and can also be part of a pressure application system, such as a sliding lid on an apparatus in which the detector is situated, that keeps the cartridge in place.

FIGS. 29-31B depict an embodiment of a highly sensitive 55 fluorescence detection system that includes light emitting diodes (LED's), photodiodes, and filters/lenses for monitoring, in real-time, one or more fluorescent signals emanating from the microfluidic channel. The embodiment in FIGS. 29-31B displays a two-color detection system having a modular design that couples with a single microfluidic channel found, for example, in a microfluidic cartridge. It would be understood by one skilled in the art that the description herein could also be adapted to create a detector that just detects a single color of light. FIGS. 31A and 31B show elements of 65 optical detector elements 1220 including light sources 1232 (for example, light emitting diodes), lenses 1234, light detec-

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tors 1236 (for example, photodiodes) and filters 1238. The detector comprises two LED's (blue and red, respectively) and two photodiodes. The two LED's are configured to transmit a beam of focused light on to a particular region of the cartridge. The two photo diodes are configured to receive light that is emitted from the region of the cartridge. One photodiode is configured to detect emitted red light, and the other photodiode is configured to detect emitted blue light. Thus, in this embodiment, two colors can be detected simultaneously from a single location. Such a detection system can be configured to receive light from multiple microfluidic channels by being mounted on an assembly that permits it to slide over and across the multiple channels. The filters can be, for example, bandpass filters, the filters at the light sources corresponding to the absorption band of one or more fluorogenic probes and the filters at the detectors corresponding to the emission band of the fluorogenic probes.

FIGS. 32 and 33 show an exemplary read-head comprising a multiplexed 2 color detection system that is configured to mate with a multi-lane microfluidic cartridge. FIG. 32 shows a view of the exterior of a multiplexed read-head. FIG. 33 is an exploded view that shows how various detectors are configured within an exemplary multiplexed read head, and in communication with an electronic circuit board.

Each of the detection systems multiplexed in the assembly of FIGS. **32** and **33** is similar in construction to the embodiment of FIGS. **29-31B**. The module in FIGS. **32** and **33** is configured to detect fluorescence from each of 12 microfluidic channels, as found in, for example, the respective lanes of a 12-lane microfluidic cartridge. Such a module therefore comprises 24 independently controllable detectors, arranged as 12 pairs of identical detection elements. Each pair of elements is then capable of dual-color detection of a pre-determined set of fluorescent probes. It would be understood by one of ordinary skill in the art that other numbers of pairs of detectors are consistent with the apparatus described herein. For example, 4, 6, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 pairs are also consistent and can be configured according to methods and criteria understood by one of ordinary skill in the

Detection Sensitivity, Time Constant and Gain

A typical circuit that includes a detector as described herein includes, in series, a preamplifier, a buffer/inverter, a filter, and a digitizer. Sensitivity is important so that high gain is very desirable. In one embodiment of the preamplifier, a very large, for example 100 G $\Omega$ , resistor is placed in parallel with the diode. Other values of a resistor would be consistent with the technology herein: such values typically fall in the range 0.5-100 G $\Omega$ , such as 1-50 G $\Omega$ , or 2-10 G $\Omega$ . An exemplary pre-amplifier circuit configured in this way is shown in FIG. 7. Symbols in the figure have their standard meanings in electronic circuit diagrams.

The FIG. 34 shows a current-to-voltage converter/pre-amplifier circuit suitable for use with the detection system. D11 is the photodetector that collects the fluorescent light coming from the microfluidic channel and converts it into an electric current. The accompanying circuitry is used to convert these fluorescent currents into voltages suitable for measurement and output as a final measure of the fluorescence.

A resistor-capacitor circuit in FIG. 34 contains capacitor C45 and resistor R25. Together, the values of capacitance of C45 and resistance of R25 are chosen so as to impact the time constant  $\tau_c$  (equal to the product of R25 and C45) of the circuit as well as gain of the detection circuit. The higher the time constant, the more sluggish is the response of the system to incident light. It typically takes the duration of a few time constants for the photodetector to completely charge to its

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maximum current or to discharge to zero from its saturation value. It is important for the photo current to decay to zero between measurements, however. As the PCR systems described herein are intended to afford rapid detection measurements, the product R<sub>25</sub>C<sub>45</sub> should therefore be made as low as possible. However, the gain of the pre-amplifier whose circuitry is shown is directly proportional to the (fluorescentactivated) current generated in the photodetector and the resistance R<sub>25</sub>. As the fluorescence signal from the microfluidic channel device is very faint (due to low liquid volume as well as small path lengths of excitation), it is thus important to maximize the value of R25. In some embodiments, R25 is as high as 100 Giga-Ohms (for example, in the range 10-100  $G\Omega$ ), effectively behaving as an open-circuit. With such values, the time-constant can take on a value of approximately 50-100 ms by using a low-value capacitor for C45. For example, the lowest possible available standard off-the-shelf capacitor has a value of 1 pF (1 picoFarad). A time-constant in the range 50-100 ms ensures that the photocurrent decays to 20 zero in approximately 0.5 s (approx. 6 cycles) and therefore that approximately 2 samplings can be made per second. Other time constants are consistent with effective use of the technology herein, such as in the range 10 ms-1 s, or in the range 50 ms-500 ms, or in the range 100-200 ms. The actual 25 time constant suitable for a given application will vary according to circumstance and choice of capacitor and resistor values. Additionally, the gain achieved by the pre-amplifier circuit herein may be in the range of  $10^7$ - $5\times10^9$ , for example may be  $1\times10^9$ .

As the resistance value for R25 is very high ( $\sim 100~\rm G\Omega$ ), the manner of assembly of this resistor on the optics board is important for the overall efficiency of the circuit. Effective cleaning of the circuit during assembly and before use is important to achieve an optimal time-constant and gain for 35 the optics circuit.

It is also important to test each photo-diode that is used, because many do not perform according to specification. Sensitivity and Aperturing

The LED light passes through a filter before passing 40 through the sample in the microfluidic channel (as described herein, typically 300 $\mu$  deep). This is a very small optical path-length for the light in the sample. The generated fluorescence then also goes through a second filter, and into a photo-detector. Ultimately, then, the detector must be capable 45 of detecting very little fluorescence. Various aspects of the detector configuration can improve sensitivity, however.

The illumination optics can be designed so that the excitation light falling on the PCR reactor is incident along an area that is similar to the shape of the reactor. As the reactor is 50 typically long and narrow, the illumination spot should be long and narrow, i.e., extended, as well. The length of the spot can be adjusted by altering a number of factors, including: the diameter of the bore where the LED is placed (the tube that holds the filter and lens has an aperturing effect); the distance 55 of the LED from the PCR reactor; and the use of proper lens at the right distance in between. As the width of the beam incident on the reactor is determined by the bore of the optical element (approximately 6 mm in diameter), it is typical to use an aperture (a slit having a width approximately equal to the 60 width of the reactor, and a length equal to the length of the detection volume) to make an optimal illumination spot. A typical spot, then, is commensurate with the dimensions of a PCR reaction chamber, for example 1.5 mm wide by 7 mm long. FIG. 35A shows the illumination spot across 12 PCR reactors for the two different colors used. A typical aperture is made of anodized aluminum and has very low autofluores38

ence in the wavelengths of interest. FIG. **35**B illustrates a cross-section of a detector, showing an exemplary location for an aperture **802**.

The optimal spot size and intensity is importantly dependent on the ability to maintain the correct position of the LED's with respect to the center of the optical axis. Special alignment procedures and checks can be utilized to optimize this. The different illuminations can also be normalized with respect to each other by adjusting the power current through each of the LED's or adjusting the fluorescence collection time (the duration for which the photodetector is on before measuring the voltage) for each detection spot. It is also important to align the detectors with the axis of the microchannels.

The aperturing is also important for successful fluorescence detection because as the cross-sectional area of the incident beam increases in size, so the background fluorescence increases, and the fluorescence attributable only to the molecules of interest (PCR probes) gets masked. Thus, as the beam area increases, the use of an aperture increases the proportion of collected fluorescence that originates only from the PCR reactor. Note that the aperture used in the detector herein not only helps collect fluorescence only from the reaction volume but it correspondingly adjusts the excitation light to mostly excite the reaction volume. The excitation and emission aperture is, of course, the same.

Based on a typical geometry of the optical excitation and emission system and aperturing, show spot sizes as small as 0.5 mm by 0.5 mm and as long as 8 mm×1.5 mm have been found to be effective. By using a long detector (having an active area 6 mm by 1 mm) and proper lensing, the aperture design can extend the detection spot to as long as 15-20 mm, while maintaining a width of 1-2 mm using an aperture. Correspondingly, the background fluorescence decreases as the spot size is decreased, thereby increasing the detection sensitivity.

Use of Detection System to Measure/Detect Fluid in PCR Chamber

The fluorescence detector is sensitive enough to be able to collect fluorescence light from a PCR chamber of a microfluidic substrate. The detector can also be used to detect the presence of liquid in the chamber, a measurement that provides a determination of whether or not to carry out a PCR cycle for that chamber. For example, in a multi-sample cartridge, not all chambers will have been loaded with sample; for those that are not, it would be unnecessary to apply a heating protocol thereto. One way to determine presence or absence of a liquid is as follows. A background reading is taken prior to filling the chamber with liquid. Another reading is taken after microfluidic operations have been performed that should result in filling the PCR chamber with liquid. The presence of liquid alters the fluorescence reading from the chamber. A programmable threshold value can be used to tune an algorithm programmed into a processor that controls operation of the apparatus as further described herein (for example, the second reading has to exceed the first reading by 20%). If the two readings do not differ beyond the programmed margin, the liquid is deemed to not have entered the chamber, and a PCR cycle is not initiated for that chamber. Instead, a warning is issued to a user.

Exemplary Electronics and Software

The heater unit described herein can be controlled by various electronics circuitry, itself operating on receipt of computer-controlled instructions. FIG. 36 illustrates exemplary electronics architecture modules for operating a heater unit and diagnostic apparatus. It would be understood by one of ordinary skill in the art that other configurations of electronics

components are consistent with operation of the apparatus as described herein. In the exemplary embodiment, the electronics architecture is distributed across two components of the apparatus: the Analyzer 2100 and a Heater unit 2102. The Analyzer apparatus as further described herein contains, for 5 example, an Optical Detection Unit 2108, a Control Board 2114, a Backplane 2112, and a LCD Touchscreen 2110. The Control Board includes a Card Engine 2116 further described herein, and Compact Flash memory 2118, as well as other components. The Heater Assembly includes a Heater Board 2104 and a Heater Mux Board 2106, both further described

In one embodiment, the Card Engine electronics module 2116 is a commercial, off the shelf "single board computer"  $_{15}$ containing a processor, memory, and flash memory for operating system storage.

The optional LCD+Touchscreen electronics module 2110 is a user interface, for example, driven through a touchscreen, such as a 640 pixel by 480 pixel 8 inch LCD and 5-wire 20

The Compact Flash electronics module 2118 is, for example, a 256 megabyte commercial, off the shelf, compact flash module for application and data storage. Other media are alternatively usable, such as USB-drive, smart media 25 circuitry that can be employed to collect, digitize, filter, and card, memory stick, and smart data-card having the same or other storage capacities.

The Backplane electronics module 2112 is a point of connection for the removable heater assembly 2102. Bare PC boards with two connectors are sufficient to provide the nec- 30 essary level of connectivity.

The Control Board electronics module 2114 supports peripherals to the Card Engine electronics module 2116. In one embodiment, the peripherals include such devices as a USB host+slave or hub, a USB CDROM interface, serial 35 ports, and ethernet ports. The Control Board 2114 can include a power monitor with a dedicated processor. The Control Board may also include a real time clock. The Control Board may further include a speaker. The Control Board 2114 also includes a CPLD to provide SPI access to all other modules 40 and programming access to all other modules. The Control Board includes a programmable high voltage power supply. The Control Board includes a Serial-Deserializer interface to the LCD+Touchscreen electronics module 2110 and to the Optical Detection Unit electronics module 2108. The Control 45 Board also includes module connectors.

In the exemplary embodiment, the optical detection unit electronics module 2108 contains a dedicated processor. The optical detection unit 2108 contains a serializer-deserializer interface. The optical detection unit 2108 contains LED driv- 50 ers. The optical detection unit also contains high gain-low noise photodiode amplifiers. The optical detection unit can have power monitoring capability. The optical detection unit can also be remotely reprogrammable.

The Heater Board electronics module 2104 is preferably a 55 glass heater board. The Heater Board has PCB with bonding pads for glass heater board and high density connectors.

In one embodiment, the heater mux ('multiplex') board electronics module 2106 has 24 high-speed ADC, 24 precision current sources, and 96 optically isolated current drivers 60 for heating. The heater mux board has the ability to timemultiplex heating/measurement. The heater mux board has multiplexer banks to multiplex inputs to ADC, and to multiplex current source outputs. The heater mux board has a FPGA with a soft processor core and SDRAM. The heater 65 mux board has a Power Monitor with a dedicated processor. The Heater Mux Board can be remotely reprogrammable.

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In another embodiment, control electronics can be spread over four different circuit board assemblies. These include the MAIN board: Can serve as the hub of the Analyzer control electronics and manages communication and control of the other various electronic subassemblies. The main board can also serve as the electrical and communications interface with the external world. An external power supply (12V DC/10A; UL certified) can be used to power the system. The unit can communicate via 5 USB ports, a serial port and an Ethernet port. Finally, the main board can incorporate several diagnostic/safety features to ensure safe and robust operation of the

MUX Board: Upon instruction from the main board, the MUX board can perform all the functions typically used for accurate temperature control of the heaters and can coordinate the collection of fluorescence data from the detector board.

LCD Board: Can contain the typical control elements to light up the LCD panel and interpret the signals from the touch sensitive screen. The LCD/touch screen combination can serve as a mode of interaction with the user via a Graphical User Interface.

Detector Board: Can house typical control and processing transmit the data from the fluorescence detection modules.

Certain software can be executed in each electronics module. The Control Board Electronics Module executes, for example, Control Board Power Monitor software. The Card Engine electronics module executes an operating system, graphical user interface (GUI) software, an analyzer module, and an application program interface (api). The Optical Detection Unit electronics module executes an optics software module. The Heater Mux Board electronics module executes dedicated Heater Mux software, and Heater Mux Power Monitor software. Each of the separate instances of software can be modular and under a unified control of, for example, driver software.

The exemplary electronics can use Linux, UNIX, Windows, or MacOS, including any version thereof, as the operating system. The operating system is preferably loaded with drivers for USB, Ethernet, LCD, touchscreen, and removable media devices such as compact flash. Miscellaneous programs for configuring the Ethernet interface, managing USB connections, and updating via CD-ROM can also be included.

In the embodiment of FIG. 36, the analyzer module is the driver for specific hardware. The analyzer module provides access to the Heater Mux Module, the Optical Detection Unit, the Control Board Power Monitor, the Real Time Clock, the High Voltage Power Supply, and the LCD backlight. The analyzer module provides firmware programming access to the Control Board power monitor, the Optical Detection Unit, and the Heater Mux Module.

The API provides uniform access to the analyzer module driver. The API is responsible for error trapping, and interrupt handling. The API is typically programmed to be thread safe.

The GUI software can be based on a commercial, off-theshelf PEG graphics library. The GUI can use the API to coordinate the self-test of optical detection unit and heater assembly. The GUI starts, stops, and monitors test progress. The GUI can also implement an algorithm to arrive on diagnosis from fluorescence data. The GUI provides access control to unit and in some embodiments has an HIS/LIS interface.

The Control Board Power Monitor software monitors power supplies, current and voltage, and signals error in case of a fault.

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The Optics Software performs fluorescence detection which is precisely timed to turn on/off of LED with synchronous digitization of the photodetector outputs. The Optics Software can also monitor power supply voltages. The Optics Software can also have self test ability.

The Heater Mux Module software implements a "protocol player" which executes series of defined "steps" where each "step" can turn on sets of heaters to implement a desired microfluidic action. The Heater Mux Module software also has self test ability. The Heater Mux Module software contains a fuzzy logic temperature control algorithm.

The Heater Mux Power Monitor software monitors voltage and current levels. The Heater Mux Power Monitor software can participate in self-test, synchronous, monitoring of the current levels while turning on different heaters.

#### **EXAMPLES**

The following are exemplary aspects of various parts and functions of the system described herein.

Additional embodiments of a cartridge are found in U.S. patent application Ser. No. 11/940,310, entitled "Microfluidic Cartridge and Method of Making Same", and filed on even date herewith, the specification of which is incorporated herein by reference.

Additional embodiments of heater units and arrays are described in U.S. patent application Ser. No. 11/940,315, entitled "Heater Unit for Microfluidic Diagnostic System" and filed on even date herewith, the specification of which is incorporated herein by reference in its entirety.

Further description of suitably configured detectors are described in U.S. patent application Ser. No. 11/940,321, filed on Nov. 14, 2007 and entitled "Fluorescence Detector for Microfluidic Diagnostic System", incorporated herein by reference.

#### Example 1

Analyzer Having Removable Heater Unit

This non-limiting example describes pictorially, various embodiments of an apparatus, showing incorporation of a heater unit and a microfluidic cartridge operated on by the heater unit.

FIG. 37 shows an apparatus 1100 that includes a housing having a display output 1102, an openable lid 1104, and a bar code reader 1106. The cartridge is positioned in a single orientation in a receiving bay under the lid, FIG. 38. The lid of the apparatus can be closed to apply pressure to the cartridge, 50 as shown in FIG. 39. The unit currently weighs about 20 lbs. and is approximately 10" wide by 16" deep by 13" high.

FIGS. **40** and **41**: The heating stage of the apparatus can be removable for cleaning, maintenance, or to replace a custom heating stage for a particular microfluidic cartridge. FIGS. **40** 55 and **41** also show how a heater unit is insertable and removable from a front access door to an analyzer apparatus.

#### Example 2

Assembly of an exemplary Heater Unit

FIG. **42**A shows an exploded view of an exemplary heater 65 unit. The unit has a top cover and a bottom cover that together enclose a Mux board (control board), a pressure support layer,

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and insulator film, and a microthermal circuit on a PCB. The last of these is the heat source that selectively heats regions of a microfluidic substrate placed in contact therewith through the top cover.

An exemplary heater substrate, FIG. 42B, consists of a photo-lithographically processed glass wafer bonded to a standard 0.100" standard FR4 printed circuit board. The glass wafer is 0.5 mm thick and is cut into a rectangle the size of ~3.5×4.25 inches. The glass substrate has numerous metal heaters and resistive temperature sensors photo-lithographically etched on the surface of the glass wafer. The substrate is aligned and bonded to the PCBoard using a compliant epoxy, ensuring flatness to within 2-3 mils over the surface of the wafer. The cured epoxy should withstand up to 120° C. for two hours minimum. Approximately 300-400 bond pads of the size of approximately 1 mm×0.25 mm, with exposed gold surfaces, are located along the two long edges of the wafer. These pads are wirebonded (ball-bonding) to corresponding pads on the PCB using 1.5 mil gold wires. Wire bonding is a threading process, standard in semiconductor FAB. Alternatively, a flip-chip method may be used, though such methods are more complicated and may warp the wafer because of thermal mismatch. Wire bonds should have good integrity and pass defined pull strength. The substrate is baked at 120° C. for two hours and then the wire bonds are encapsulated by a compliant epoxy that will protect the wirebonds but not damage the bonds even at 120° C. Encapsulant should not spill over predefined area around the wirebonds and should not be taller than a defined height. For example, instead of laying epoxy all over the substrate, lines (e.g., a hash pattern) of it are made so that epoxy cures and air escapes through side. Alternatively, a laminate fill (adhesive on both sides) can be used. Standard connectors are soldered to the PCB and then the unit is tested using a test set-up to ensure all heaters and sensors read the right resistance values.

Pictures of an exemplary Mux board and assembled heater unit are shown in FIGS. 27-29.

#### Example 3

#### Pulse Width Modulation for Heater Circuitry

In various embodiments, the operation of a PWM generator can also include a programmable start count in addition to the aforementioned end count and granularity. In such embodiments, multiple PWM generators can produce signals that can be selectively non-overlapping (e.g., by multiplexing the on-time of the various heaters) such that the current capacity of the high voltage power is not exceeded. Multiple heaters can be controlled by different PWM signal generators with varying start and end counts. The heaters can be divided into banks, whereby a bank defines a group of heaters of the same start count. For example, 36 PWM generators can be grouped into six different banks, each corresponding to a certain portion of the PWM cycle (500 ms for this example). The end count for each PWM generator can be selectively programmed such that not more than six heaters will be on at any given time. A portion of a PWM cycle can be selected as dead time (count 3000 to 4000 for this example) during which no heating takes place and sensitive temperature sensing circuits can use this time to sense the temperature. The table below represents a PWM cycle for the foregoing example:

#### Example 4

#### Detector Integrated in Force Member

This non-limiting example describes pictorially, various embodiments of a detection system integrated into a force member, in an apparatus for carrying out diagnostics on microfluidic samples.

FIG. 43A: The lid of the apparatus can be closed, which can  $_{45}$  block ambient light from the sample bay, and place an optical detector contained in the lid into position with respect to the microfluidic cartridge.

FIG. 43B: The lid of the apparatus can be closed to apply pressure to the cartridge. Application of minimal pressure on 50 the cartridge: after the slider compresses the cartridge, the slider can compress the compliant label of the cartridge. This can cause the bottom of the cartridge to be pressed down against the surface of the heater unit present in the heater module. Springs present in the slider can deliver, for example 55 approximately 50 lb of pressure to generate a minimum pressure, for example 2 psi over the entire cartridge bottom.

Thermal interface: the cartridge bottom can have a layer of mechanically compliant heat transfer laminate that can enable thermal contact between the microfluidic substrate 60 and the microheater substrate of the heater module. A minimal pressure of 1 psi can be employed for reliable operation of the thermal valves, gate and pumps present in the microfluidic cartridge.

Mechanicals and assembly: the Analyzer can have a simple 65 mechanical frame to hold the various modules in alignment. The optics module can be placed in rails for easy opening and

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placement of cartridges in the Analyzer and error-free alignment of the optics upon closing. The heater/sensor module can be also placed on rails or similar guiding members for easy removal and insertion of the assembly.

Slider: the slider of the Analyzer can house the optical detection system as well as the mechanical assembly that can enables the optics jig to press down on the cartridge when the handle of the slider is turned down onto the analyzer. The optics jig can be suspended from the case of the slider at 4 points. Upon closing the slider and turning the handle of the analyzer down, 4 cams can turn to push down a plate that presses on 4 springs. On compression, the springs can deliver approximately 50 lb on the optical block. See FIGS. 44A-44C.

The bottom surface of the optics block can be made flat to within 100 microns, typically within 25 microns, and this flat surface can press upon the compliant (shore hardness approximately 50-70) label (approximately 1.5 mm thick under no compression) of the cartridge making the pressure more or less uniform over the cartridge. An optional lock-in mechanism can also be incorporated to prevent the slider from being accidentally knocked-off while in use.

FIG. 45A shows a side view of a lever assembly 1200, with lever 1210, gear unit 1212, and force member 1214. Assembly 1200 can be used to close the lid of the apparatus and (through force members 1214) apply force to a microfluidic cartridge 1216 in the receiving chamber 1217. One force member is visible in this cut away view, but any number, for example 4, can be used. The force members can be, for example, a manual spring loaded actuator as shown, an automatic mechanical actuator, a material with sufficient mechanical compliance and stiffness (e.g., a hard elastomeric plug), and the like. The force applied to the microfluidic cartridge 1216 can result in a pressure at the surface of the microfluidic cartridge 1216 of at least about 0.7 psi to about 7 psi (between about 5 and about 50 kilopascals), or in some embodiments about 2 psi (about 14 kilopascals).

FIG. 45B shows a side view of lever assembly 1200, with microfluidic cartridge 1216 in the receiving chamber 1217. A heat source 1219 (for example, a xenon bulb as shown) can function as a radiant heat source directed at a sample inlet reservoir 1218, where the heat can lyse cells in reservoir 1218. A thermally conductive, mechanically compliant layer 1222 can lie at an interface between microfluidic cartridge 1216 and thermal stage 1224. Typically, microfluidic cartridge 1216 and thermal stage 1224 can be planar at their respective interface surfaces, e.g., planar within about 100 microns, or more typically within about 25 microns. Layer 1222 can improve thermal coupling between microfluidic cartridge 1216 and thermal stage 1224. Optical detector elements 1220 can be directed at the top surface of microfluidic cartridge 1216.

FIGS. 45C and 45D show further cross-sectional views.

#### Example 6

#### **Exemplary Optics Board**

An exemplary optics board is shown schematically in FIG. **46**, and is used to collect and amplify the fluorescent signature of a successful chemical reaction on a micro-fluidic cartridge, and control the intensity of LED's using pulse-width modulation (PWM) to illuminate the cartridge sample over up to four channels, each with two color options. Additionally, it receives instructions and sends results data back over an LVDS (low-voltage differential signaling) SPI (serial periph-

eral interface). In some embodiments there is a separate instance of this circuitry for each PCR channel that is monitored

The power board systems include: a +12V input; and +3.3V, +3.6V, +5V, and -5V outputs, configured as follows: 5 the +3.3V output contains a linear regulator, is used to power the LVDS interface, should maintain a +/-5% accuracy, and supply an output current of 0.35 A; the +3.6V output contains a linear regulator, is used to power the MSP430, should maintain a  $\pm -5\%$  accuracy, and supply an output current of 0.35 A; 10 the +5V output contains a linear regulator, is used to power the plus rail for op-amps, should maintain a+/-5% accuracy, and supply an output current of 0.35 A; the -5V output receives its power from the +5V supply, has a mV reference, is used to power the minus rail for op-amps and for the 15 photo-detector bias, should maintain a +/-1% voltage accuracy, and supply an output current of 6.25 mA+/-10%. Additionally, the power board has an 80 ohm source resistance, and the main board software can enable/disable the regulator outputs.

The main board interface uses a single channel of the LVDS standard to communicate between boards. This takes place using SPI signaling over the LVDS interface which is connected to the main SPI port of the control processor. The interface also contains a serial port for in-system program- 25 ming.

The optical detection system of FIG. 46 comprises a control processor, LED drivers, and a photo-detection system. In the exemplary embodiment, the control processor is a TI MSP430F1611 consisting of a dual SPI (one for main board 30 interface, and one for ADC interface) and extended SRAM for data storage. It has the functions of power monitoring, PWM LED control, and SPI linking to the ADC and main board. The LED drivers contain NPN transistor switches, are connected to the PWM outputs of the control processor, can 35 sink 10 mA@12V per LED (80 mA total), and are single channel with 2 LEDs (one of each color) connected to each. The photo-detection system has two channels and consists of a photo-detector, high-sensitivity photo-diode detector, high gain current to voltage converter, unity gain voltage inverting 40 amplifier, and an ADC. Additionally it contains a 16 channel Sigma-delta (only utilizing the first 8 channels) which is connected to the second SPI port of the control processor.

During assembly of the various components on to the PC board, such as may occur on a production line, there are the 45 following considerations. The extremely high impedance of the photo-detection circuit means that a rigorous cleaning procedure must be employed. Such a procedure may include, for example: After surface mount components are installed, the boards are washed on a Weskleen and blow dried upon 50 exiting conveyor. The belt speed can be set at 20-30. The boards are soaked in an alcohol bath for approximately 3 minutes, then their entire top and bottom surfaces are scrubbed using a clean, soft bristle brush. The boards are baked in a 105° F. (40° C.) oven for 30 minutes to dry out all 55 components.

After all the components are installed: the soldered areas of the boards can be hand wash using deionized water and a soft bristle brush. The same soldered areas can be hand washed using alcohol and a soft bristle brush. The boards are allowed 60 to air dry. Once the board is cleaned, the optical circuitry must be conformal coated to keep contaminates out.

The foregoing description is intended to illustrate various aspects of the present technology. It is not intended that the examples presented herein limit the scope of the present 65 technology. The technology now being fully described, it will be apparent to one of ordinary skill in the art that many

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changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

We claim:

- 1. An apparatus, comprising:
- a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone;
- a plurality of receiving bays, each receiving bay configured to receive one of the plurality of microfluidic cartridges;
- each PCR reaction zone comprising a separately controllable heat source thermally coupled thereto, wherein the heat source thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and maintains a substantially uniform temperature throughout the PCR reaction zone during each cycle;
- a detector configured to detect the presence of an amplification product in one or more PCR reaction zones; and
- a processor coupled to the detector and the heat sources, configured to control heating of one or more PCR reaction zones by the heat sources.
- 2. The apparatus of claim 1, wherein the separately controllable heat source is configured to maintain a temperature gradient of less than 1° C. across a width of the PCR reaction zone at any point along a length of the PCR reaction zone.
- 3. The apparatus of claim 1, wherein the processor is programmable to operate the detector to detect a polynucleotide or a probe thereof in a plurality of microfluidic cartridges located in the plurality of receiving bays.
- **4.** The apparatus of claim **1**, wherein the detector comprises an optical detector, the optical detector comprising a light source that selectively emits light in an absorption band of a fluorescent dye and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof.
- 5. The apparatus of claim 4, wherein the optical detector comprises a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye.
- **6**. The apparatus of claim **1**, wherein the heat source comprises a plurality of heaters configured to maintain a substantially uniform temperature throughout a PCR reaction chamber thermally coupled to the heat source.
- 7. A device for carrying out PCR on a plurality of samples, the device comprising:
  - a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone;
  - a plurality of receiving bays, each receiving bay configured to receive one of the plurality of microfluidic cartridges;
  - a separately controllable heat source thermally coupled to each PCR reaction zone, wherein the heat source is configured to thermal cycle the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and to maintain a substantially uniform temperature throughout the PCR reaction zone during each cycle;
  - a detector configured to detect the presence of an amplification product in one or more PCR reaction zones;
  - a processor coupled to the detector and a plurality of the separately controllable heat sources, configured to control heating of one or more PCR reaction zones by one or more of the plurality of separately controllable heat sources; and
  - an input device coupled to the processor and configured to permit concurrent or consecutive control of the plurality of multi-lane microfluidic cartridges.

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- 8. The device of claim 7, wherein at least one of the plurality of separately controllable heat sources is a first contact heat source selected from a resistive heater, a radiator, a fluidic heat exchanger, and a Peltier device.
- 9. The device of claim 8, wherein the first contact heat 5 source is thermally coupled to a distinct location in a first microfluidic cartridge received in a first receiving bay, whereby the distinct location is selectively heated.
- 10. The device of claim 9, wherein the distinct location has a surface area of between about 1 mm<sup>2</sup> and about 225 mm<sup>2</sup>. 10
- 11. The device of claim 8, further comprising a second contact heat source configured to be independently thermally coupled to a distinct location in a second microfluidic cartridge received in a second receiving bay, whereby the distinct location in the second microfluidic cartridge is independently 15 samples, the method comprising: heated from the distinct location in the first microfluidic car-
- 12. The device of claim 8, wherein the first contact heat source is configured to be in direct physical contact with the distinct location of the first microfluidic cartridge received in 20 the first receiving bay.
- 13. The device of claim 8, further comprising a compliant layer configured to thermally couple the first contact heat source with at least a portion of the first microfluidic cartridge received in the first receiving bay.
- 14. The device of claim 7, wherein at least one of the plurality of separately controllable heat sources is a radiative heat source configured to direct heat to a distinct location of a first microfluidic cartridge received in a first receiving bay.
- 15. The device of claim 7, wherein the input device is 30 selected from the group consisting of a keyboard, a touchsensitive surface, a microphone, a hard disk drive, an optical disk drive, a serial connection, a parallel connection, a wireless network connection, a wired network connection, and a mouse.
- 16. The device of claim 7, further comprising at least one sample identifier coupled to the processor, the sample identifier being selected from an optical character reader, a bar code reader, and a radio frequency tag reader.

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- 17. The device of claim 7, further comprising at least one output coupled to the processor, the output being selected from a display, a printer, a speaker, a serial connection, a parallel connection, a wireless network connection, and a wired network connection.
- 18. The device of claim 6, further comprising a heating stage configured to be removable from the device, wherein at least one of the plurality of separately controllable heat sources is located in the heating stage.
- 19. The device of claim 7, wherein the heat source comprises a plurality of heaters configured to maintain a substantially uniform temperature throughout a PCR reaction chamber thermally coupled to the heat source.
- 20. A method of carrying out PCR on a plurality of
  - introducing the plurality of samples into a plurality of multi-lane microfluidic cartridges, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples:
  - moving the plurality of samples into the respective plurality of PCR reaction zones; and
  - amplifying polynucleotides contained with the plurality of samples in the plurality of PCR reaction zones while thermal cycling the PCR reaction zones and maintaining a substantially uniform temperature throughout each PCR reaction zone during each cycle, at least one PCR reaction zone separately thermally controllable from another PCR reaction zone.
- 21. The method of claim 20, further comprising detecting the presence of a polynucleotide or a polynucleotide probe in the plurality of samples.
- 22. The method of claim 20, wherein thermal cycling the PCR reaction zones comprises heating each PCR reaction 35 zone with a plurality of heaters configured to maintain a substantially uniform temperature throughout each PCR reaction zone.

# EXHIBIT 5



### (12) United States Patent Handique

## (10) **Patent No.:**

#### US 8,415,103 B2 (45) Date of Patent: \*Apr. 9, 2013

#### (54) MICROFLUIDIC CARTRIDGE

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This patent is subject to a terminal dis-

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- Division of application No. 12/239,537, filed on Sep. 26, 2008, now Pat. No. 8,105,783, which is a continuation-in-part of application No. 12/173,023, filed on Jul. 14, 2008, now Pat. No. 8,133,671, and a continuation-in-part of application No. 11/985,577, filed on Nov. 14, 2007, now Pat. No. 7,998,708, and a continuation-in-part of application No. 29/308,920, filed on Jul. 14, 2008, now Pat. No. Des. 621,060.
- Provisional application No. 60/959,437, filed on Jul. 13, 2007.
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- (52) **U.S. Cl.** ...... 435/6.12; 435/6.1; 435/6.11
- (58) Field of Classification Search ...... None See application file for complete search history.

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#### (57)ABSTRACT

The technology described herein generally relates to microfluidic cartridges configured to amplify and detect polynucleotides extracted from multiple biological samples in parallel. The technology includes a microfluidic substrate, comprising: a plurality of sample lanes, wherein each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another: an inlet; a first valve and a second valve; a first channel leading from the inlet, via the first valve, to a reaction chamber; and a second channel leading from the reaction chamber, via the second valve, to a vent.

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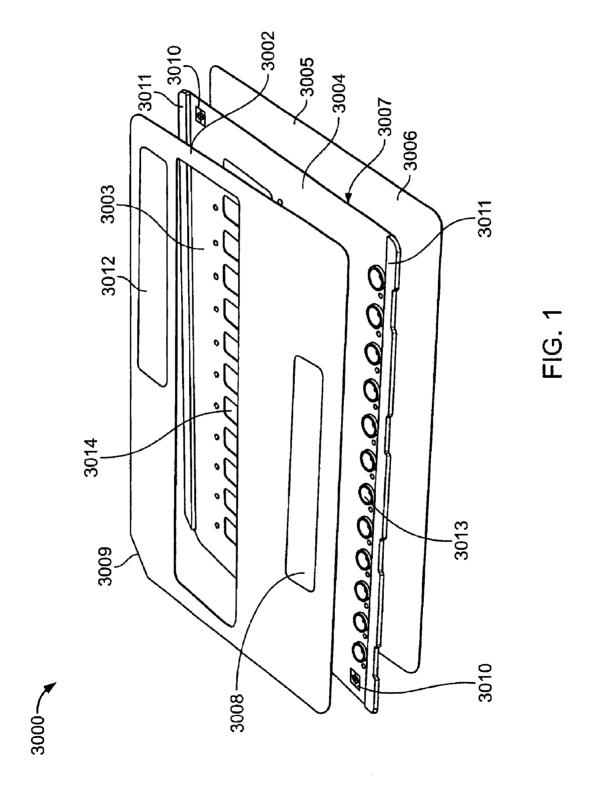
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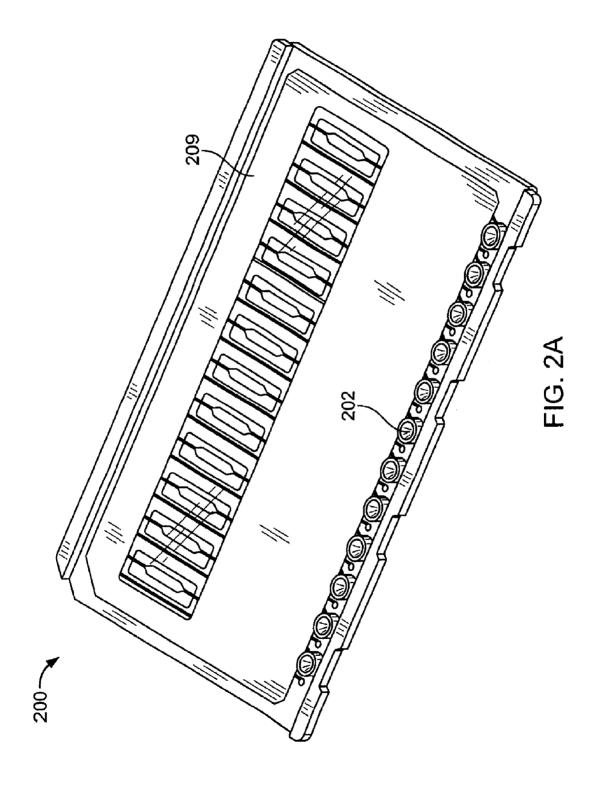
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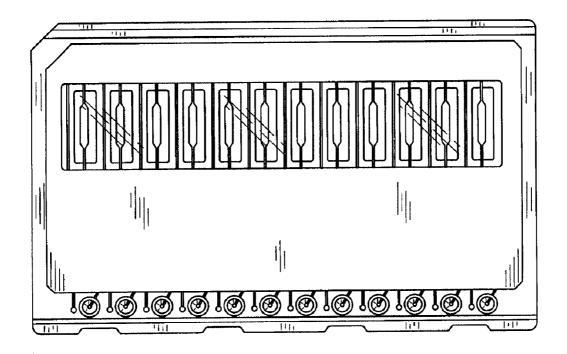


FIG. 2B

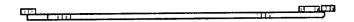
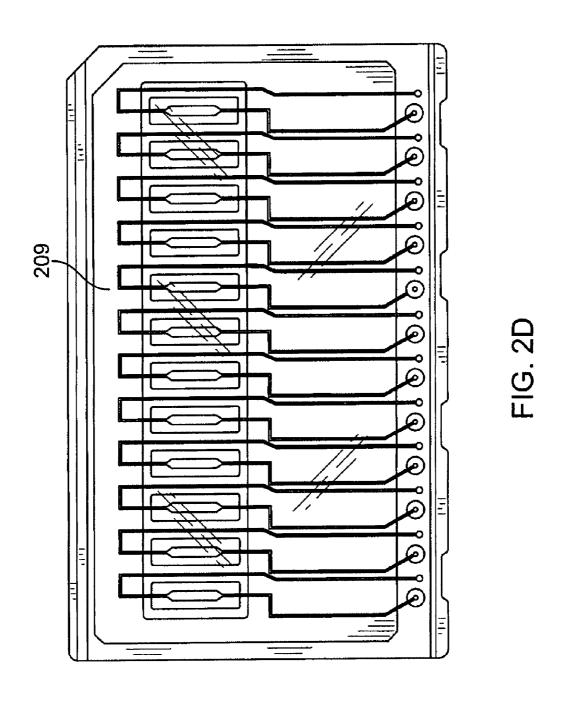


FIG. 2C

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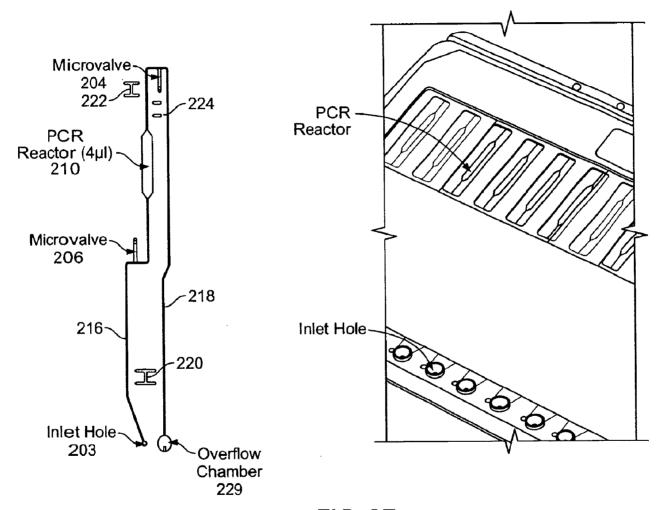
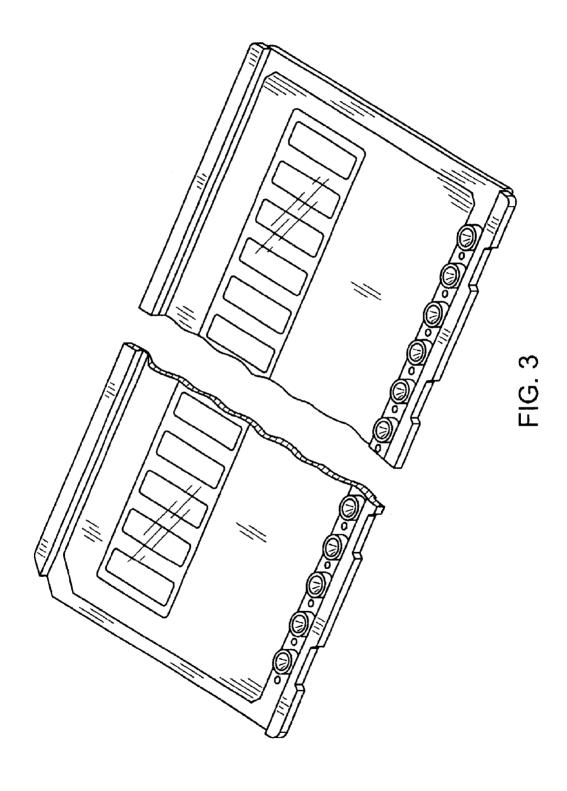


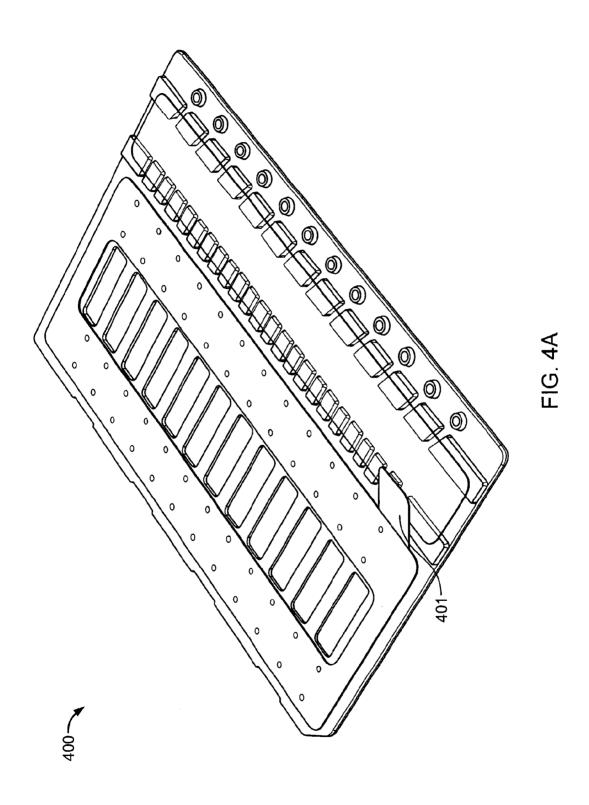
FIG. 2E

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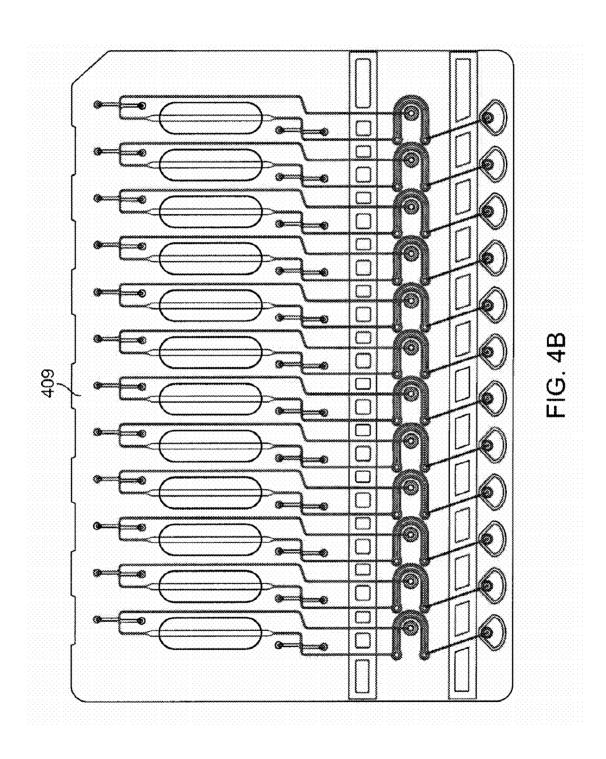
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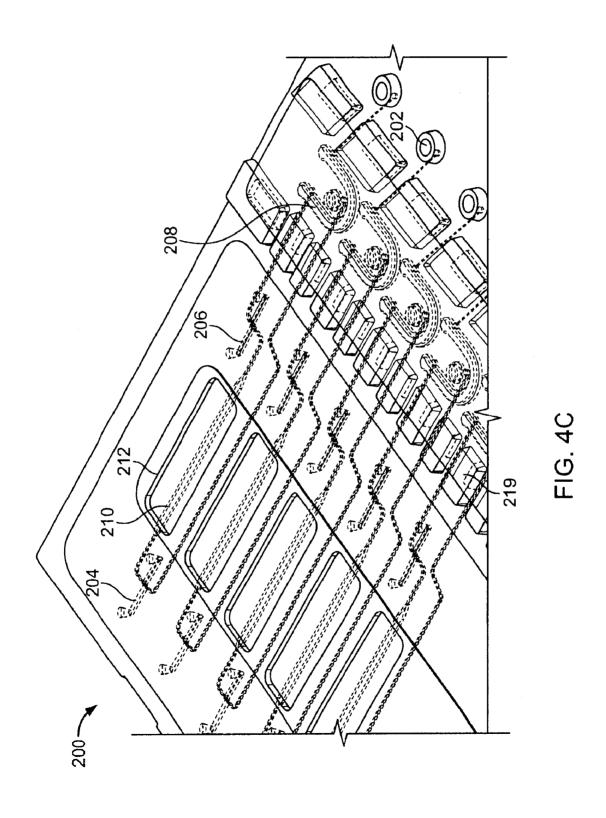


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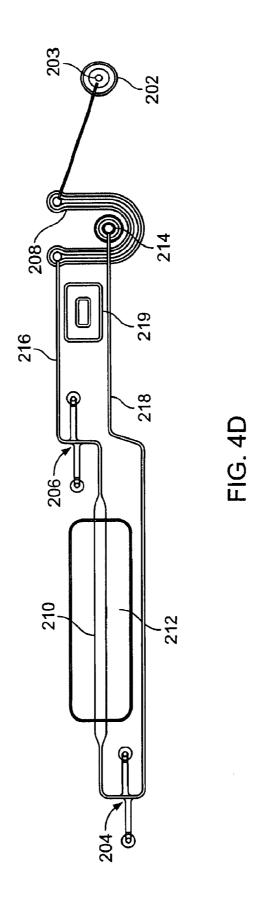
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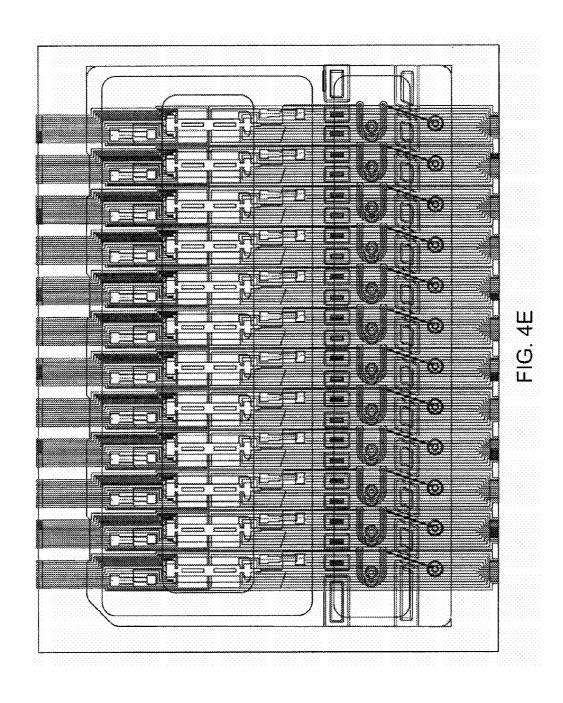
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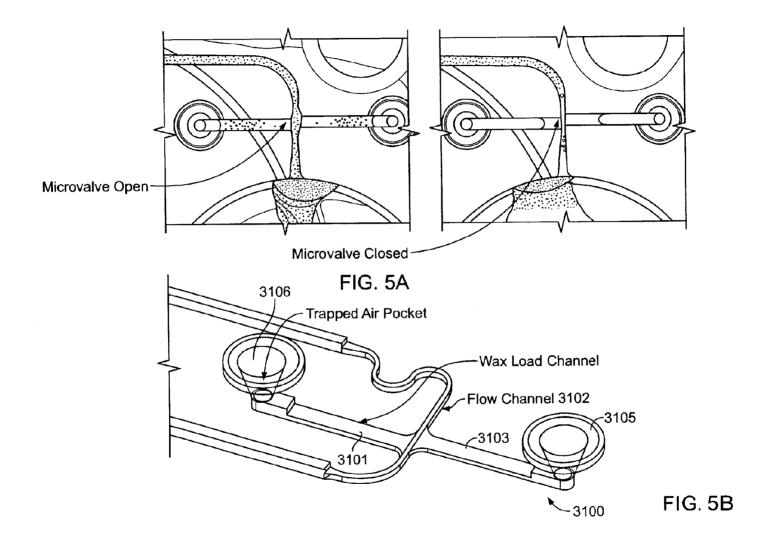
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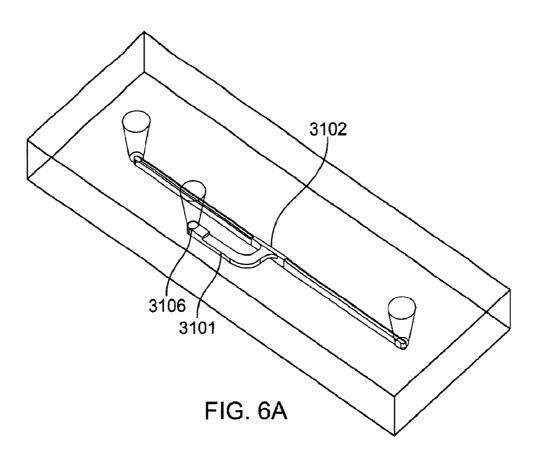
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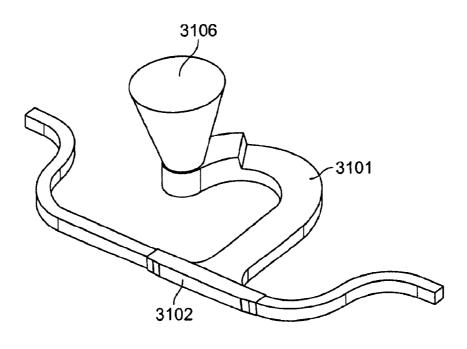
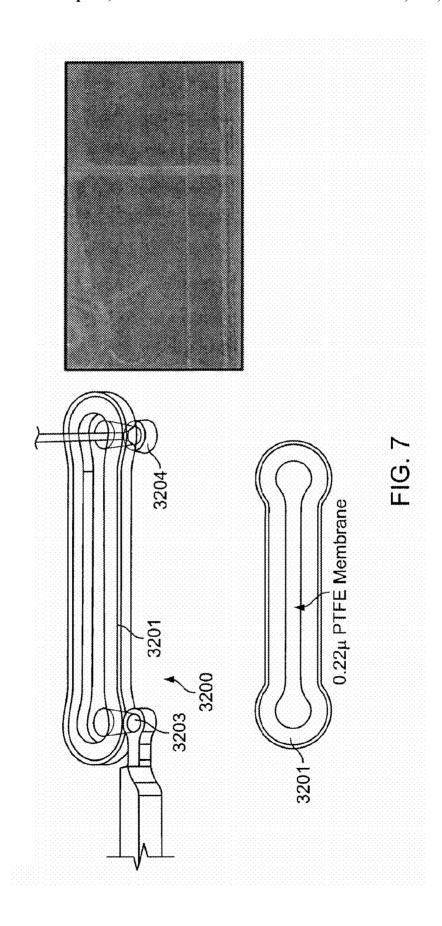


FIG. 6B

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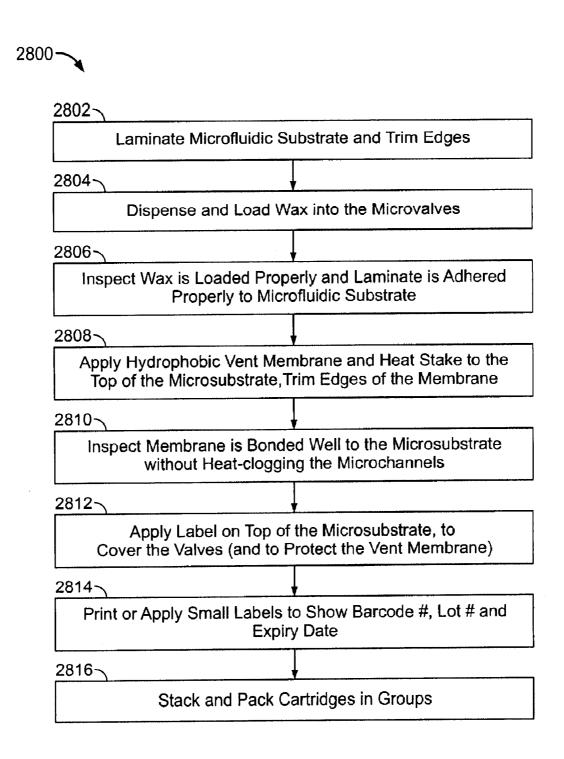


FIG. 8

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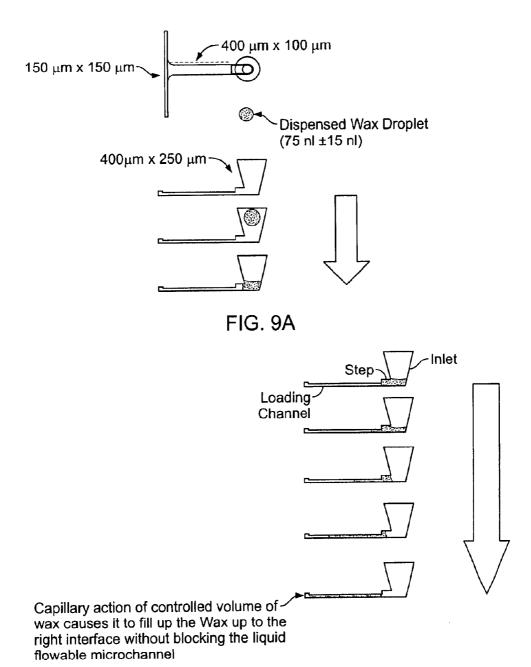


FIG. 9B

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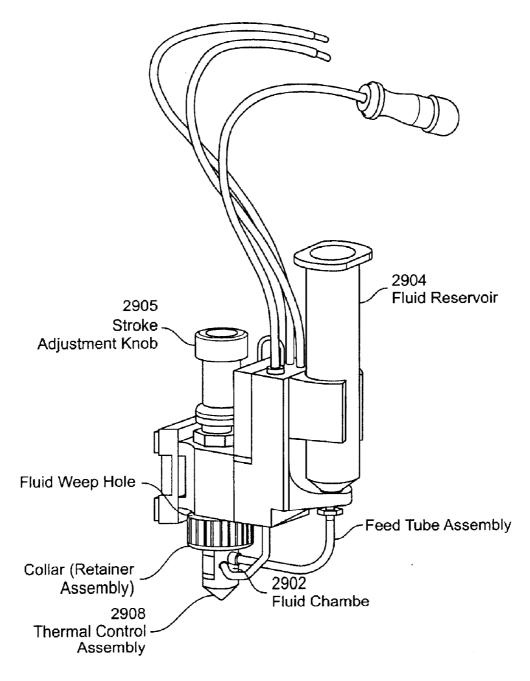


FIG. 10A

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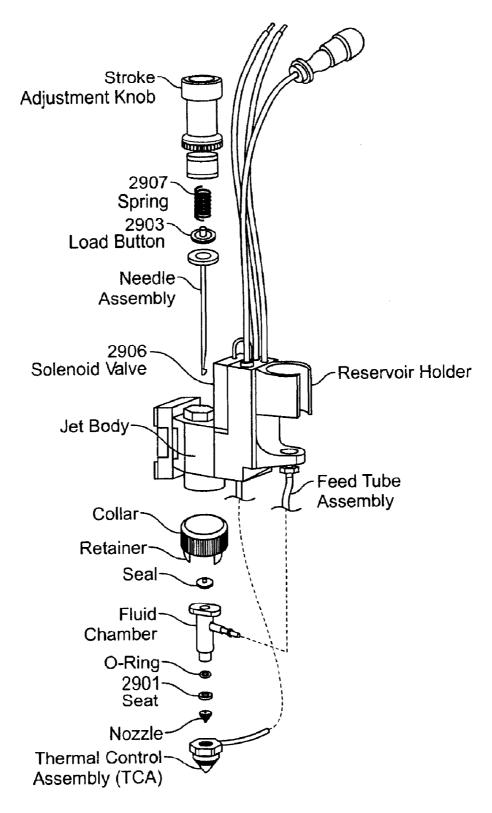
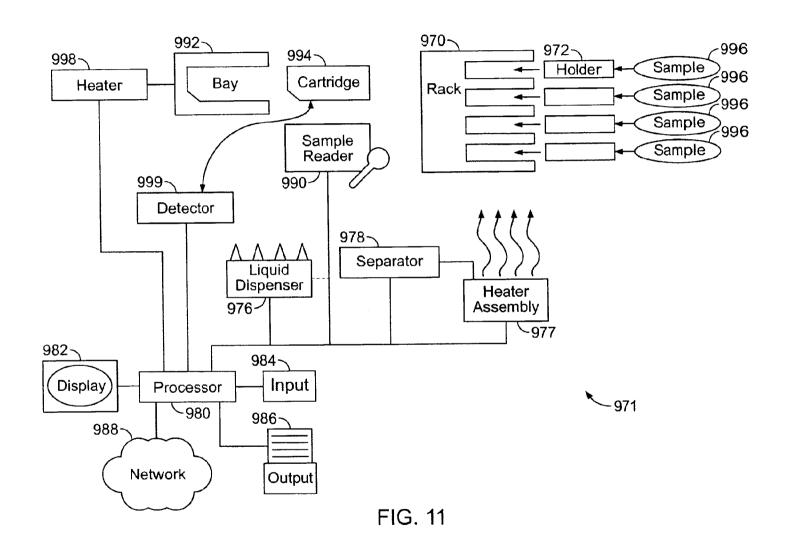


FIG. 10B



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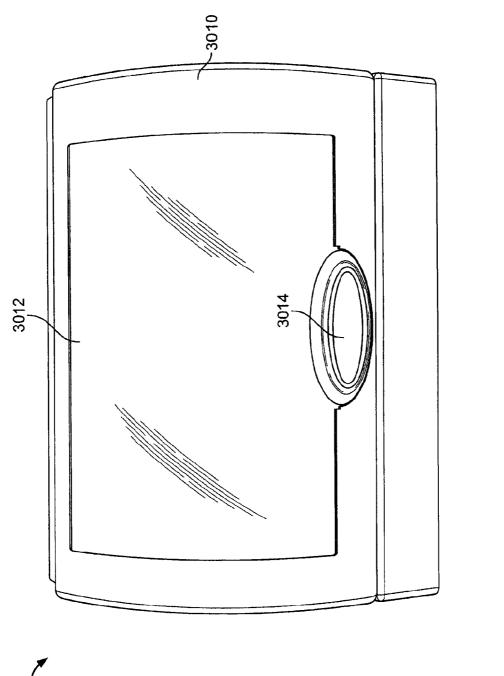
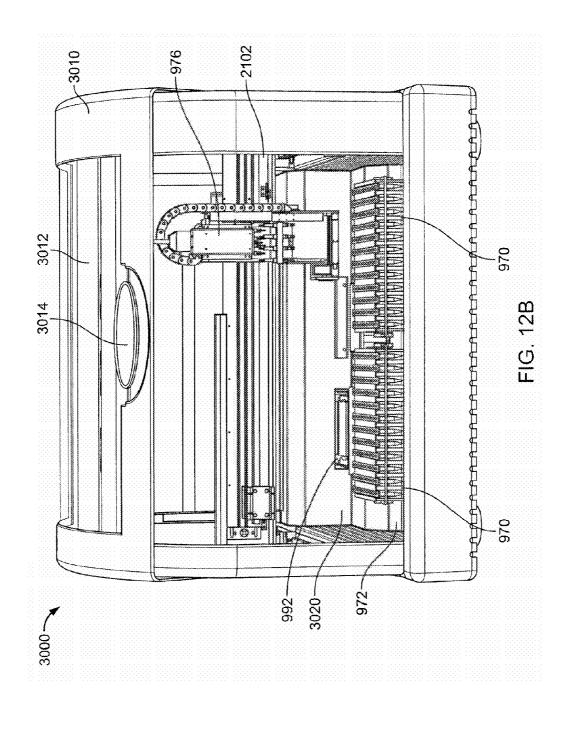


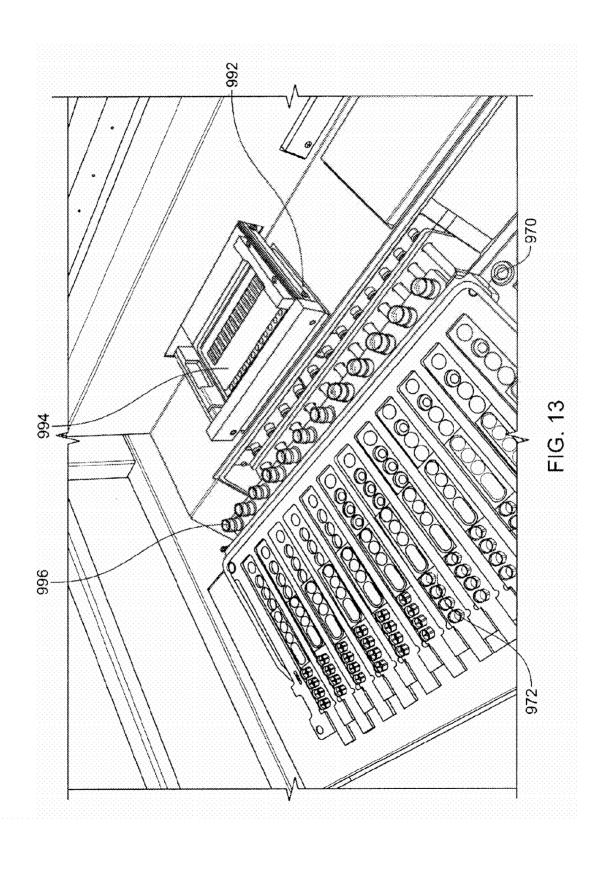
FIG. 12A

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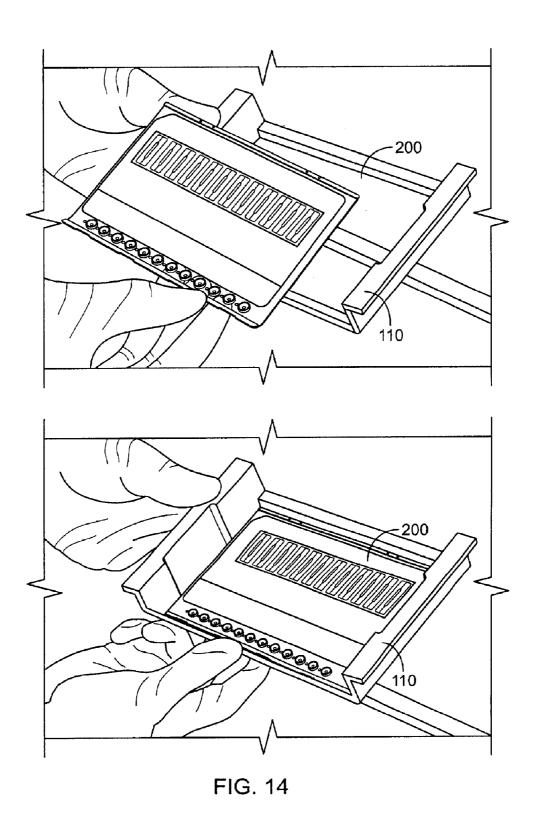


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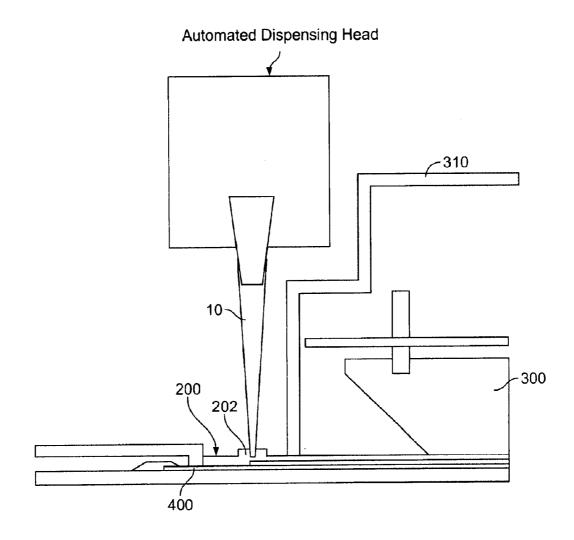
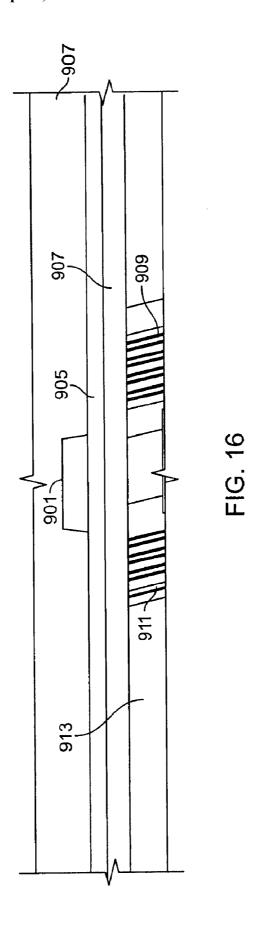


FIG. 15

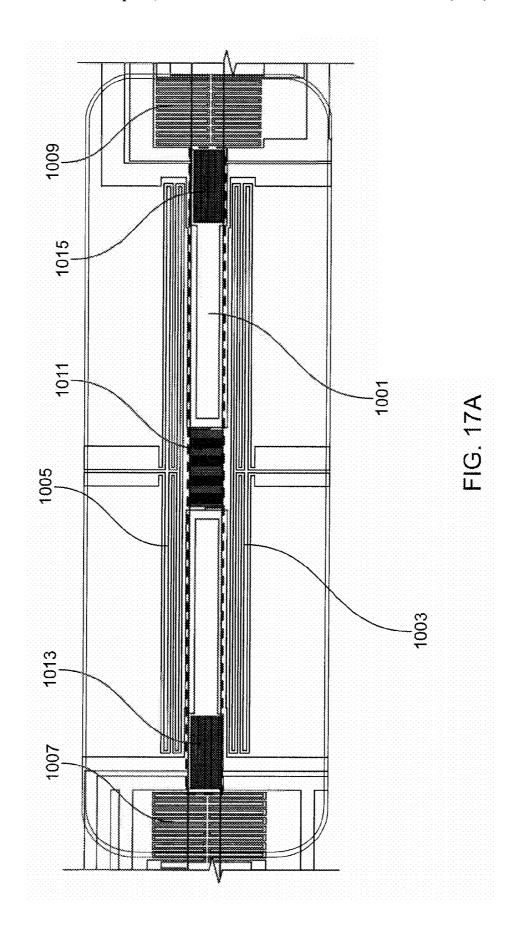
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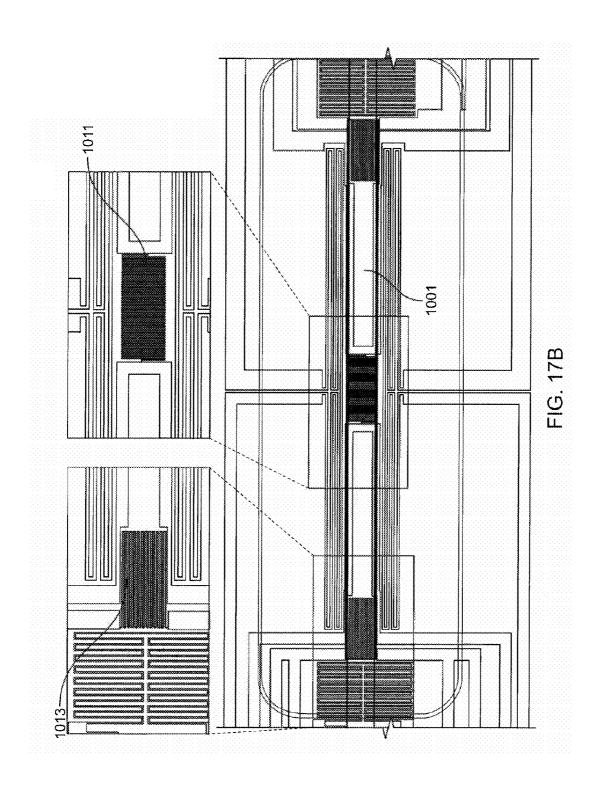
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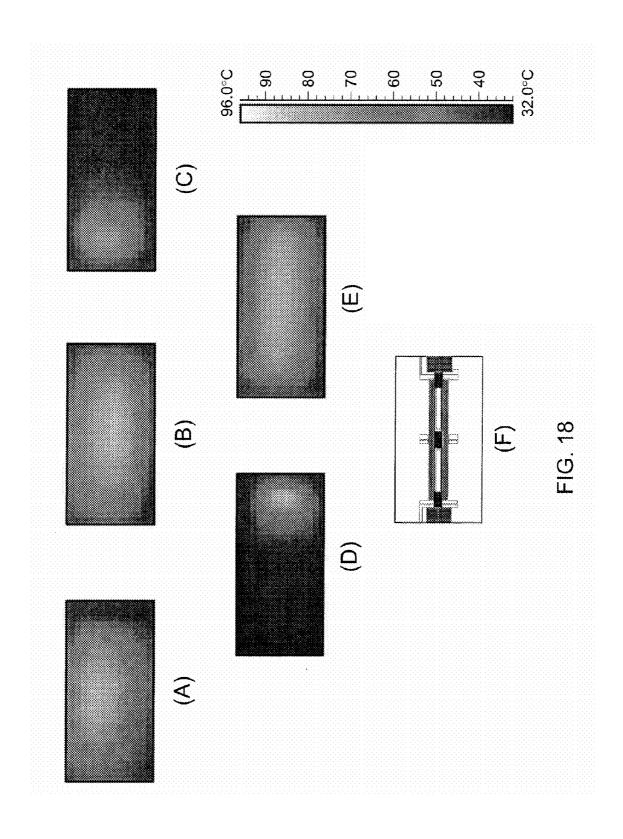
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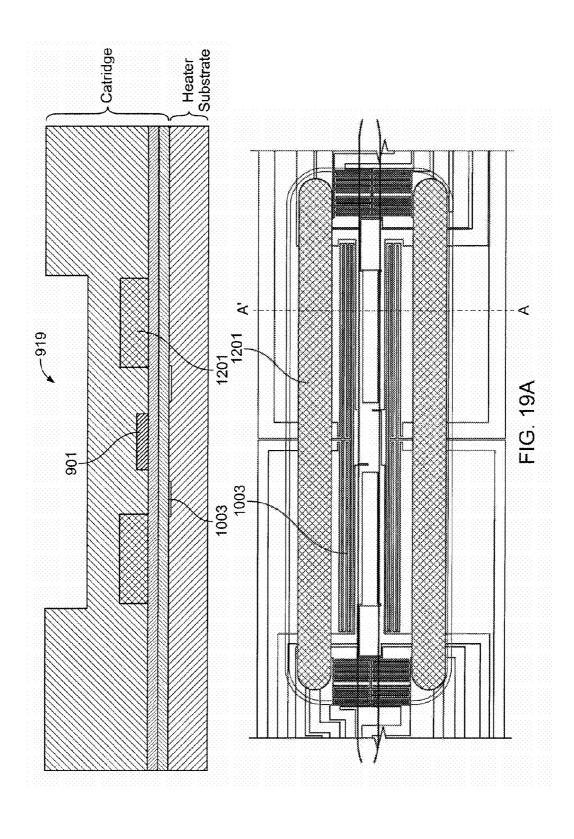
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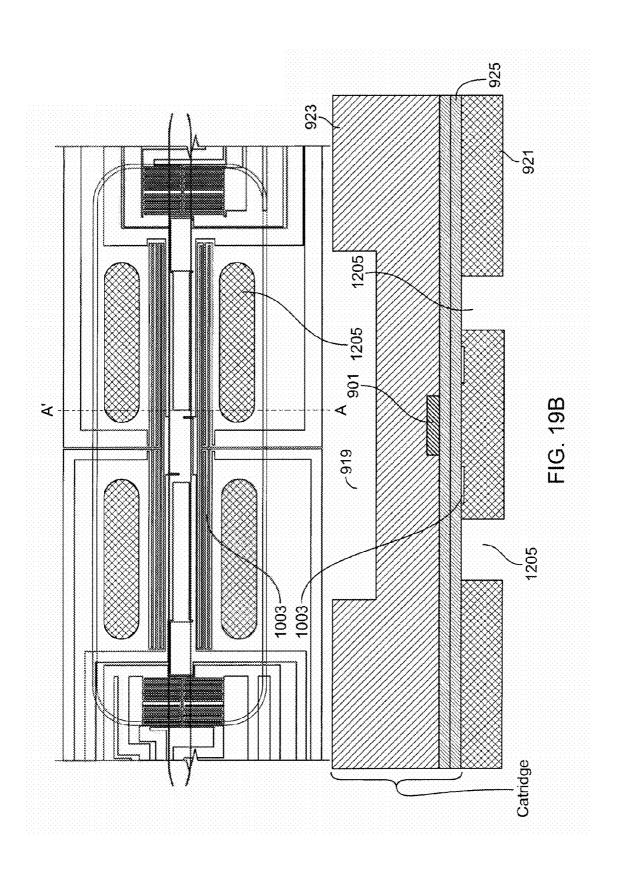
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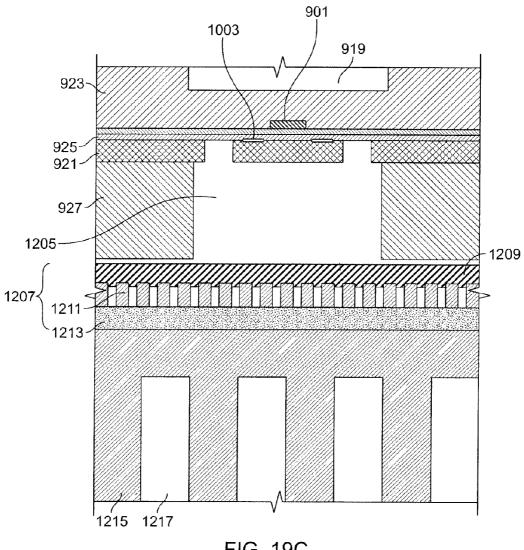
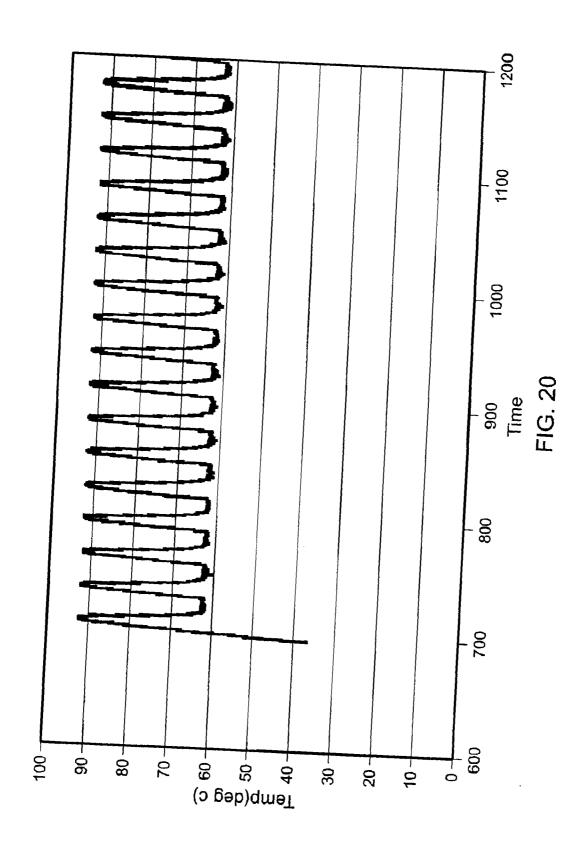


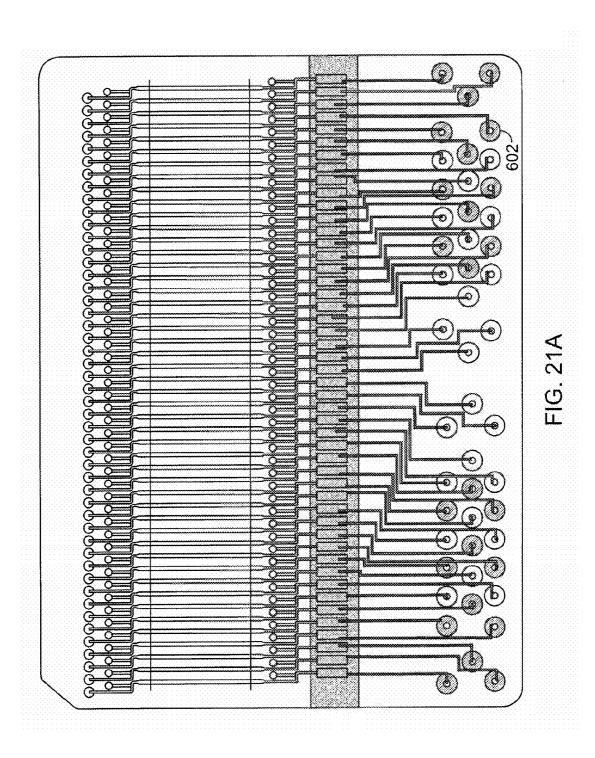
FIG. 19C

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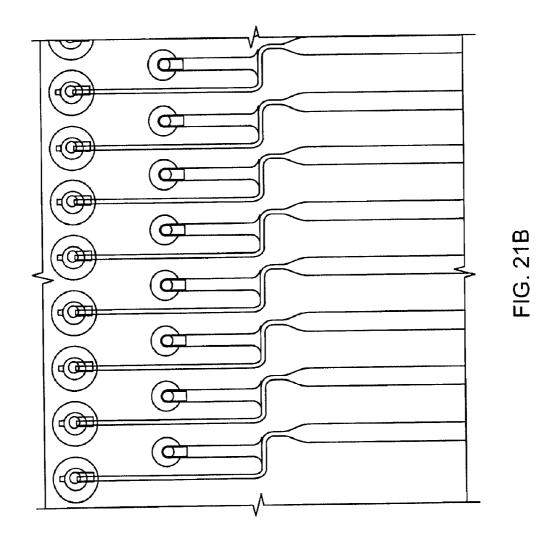


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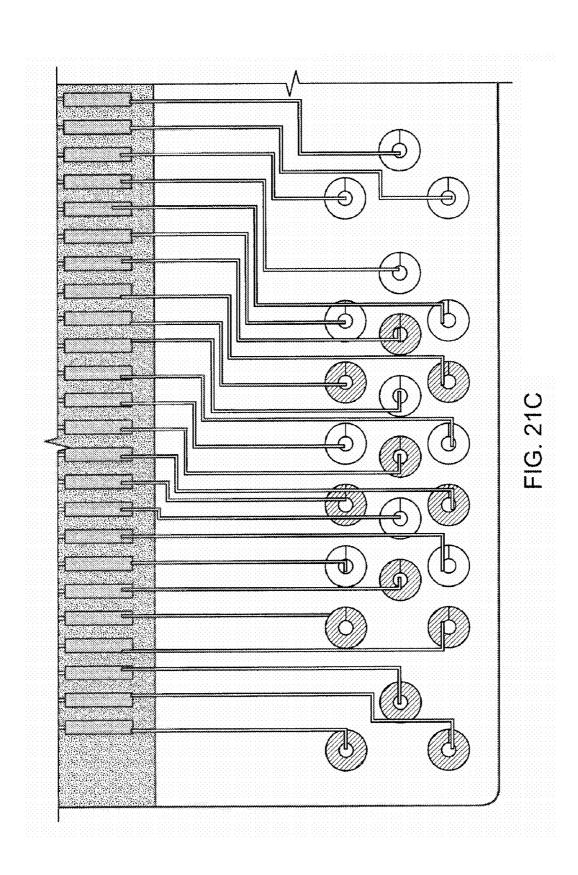
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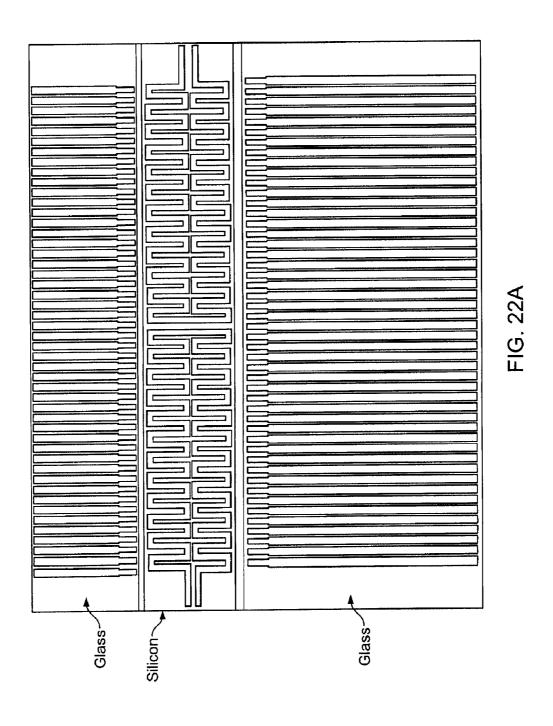
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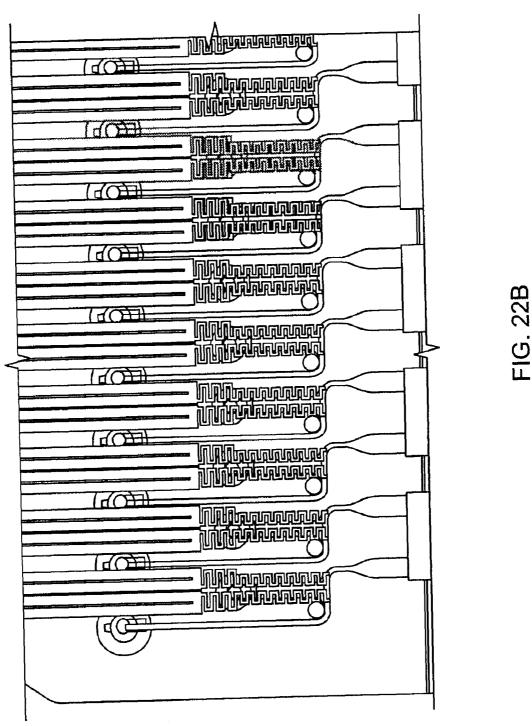
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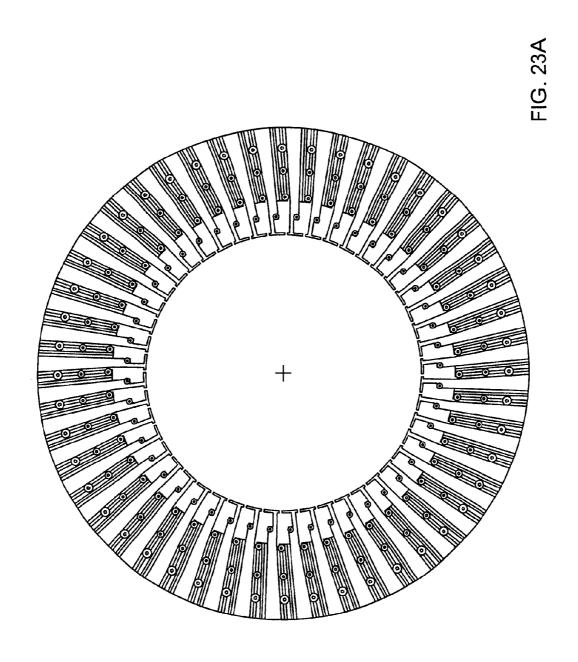
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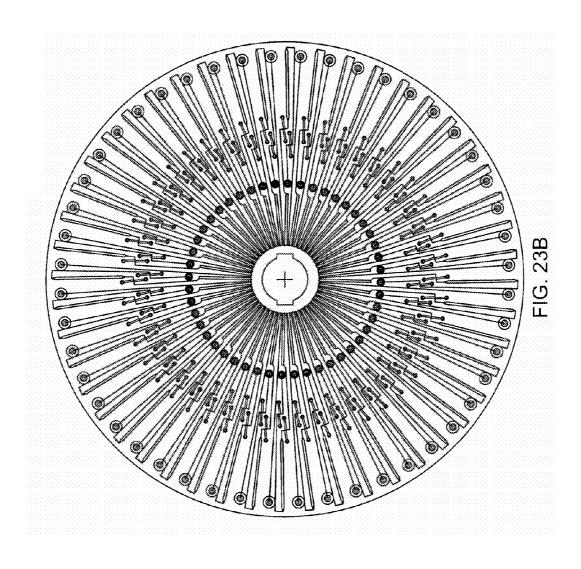
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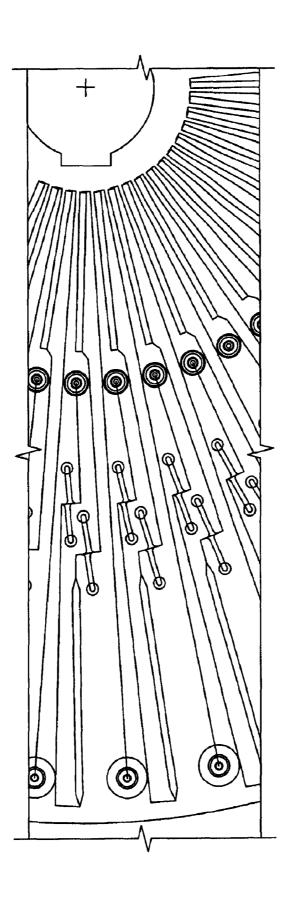
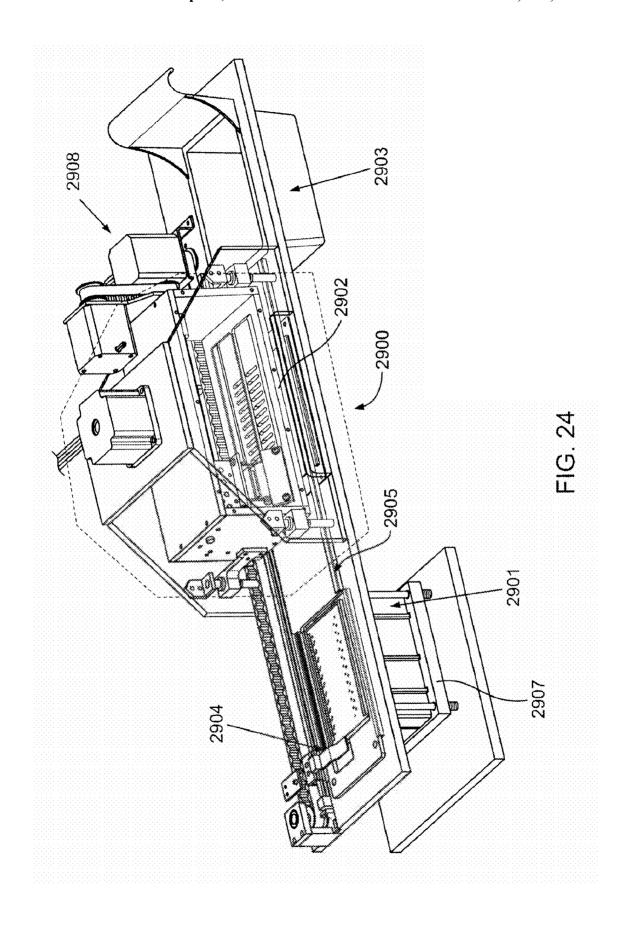


FIG. 23C

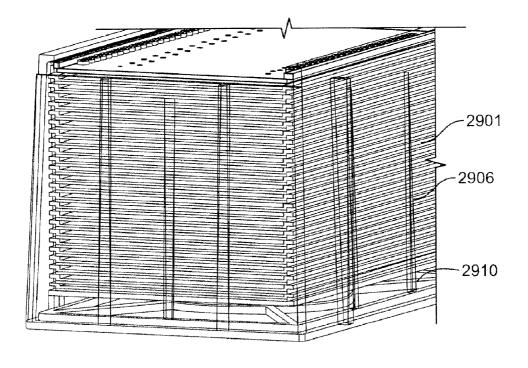
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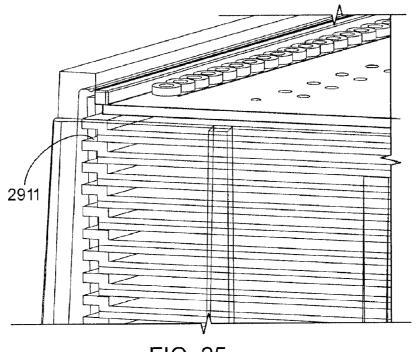
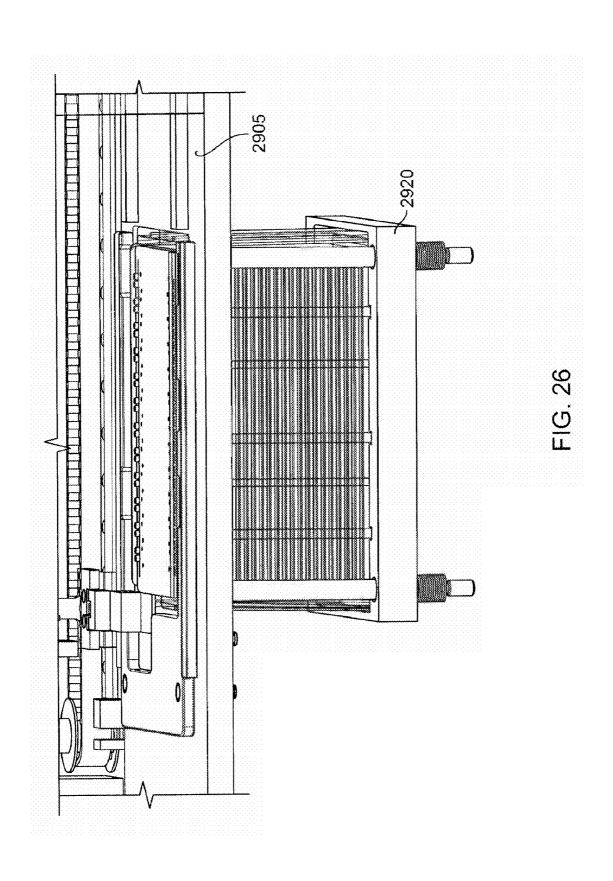


FIG. 25

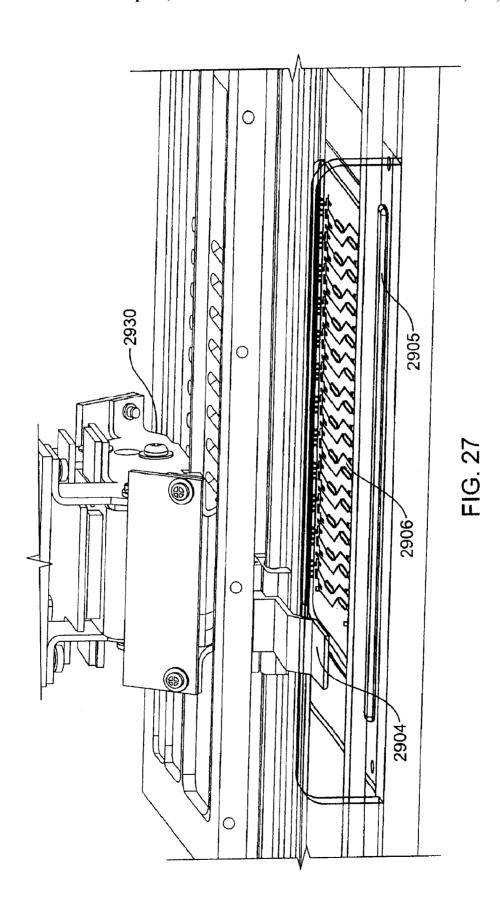
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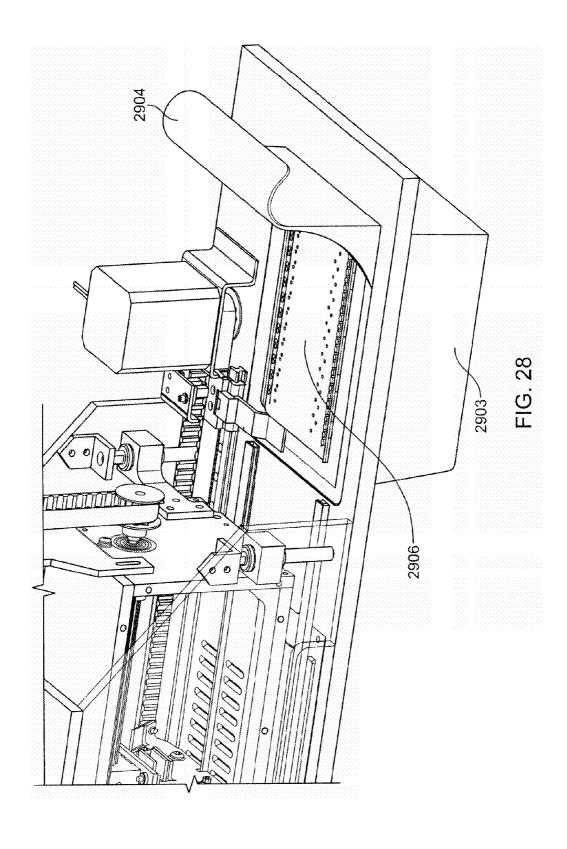
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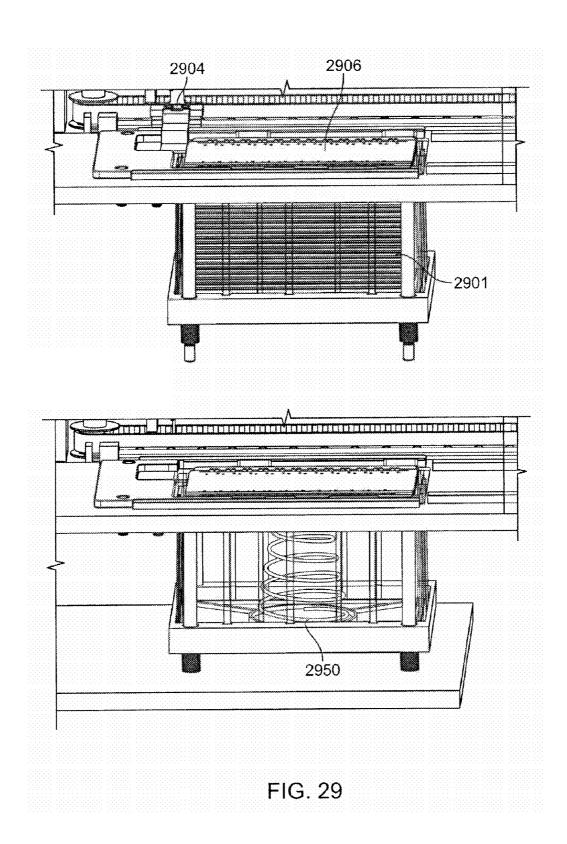
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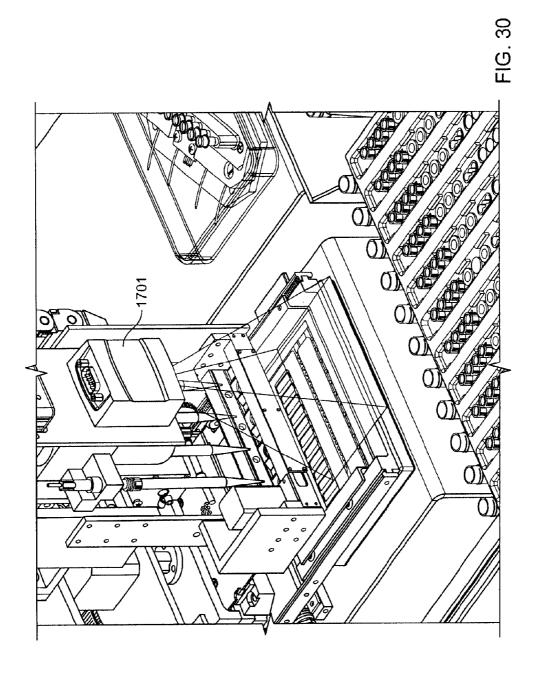


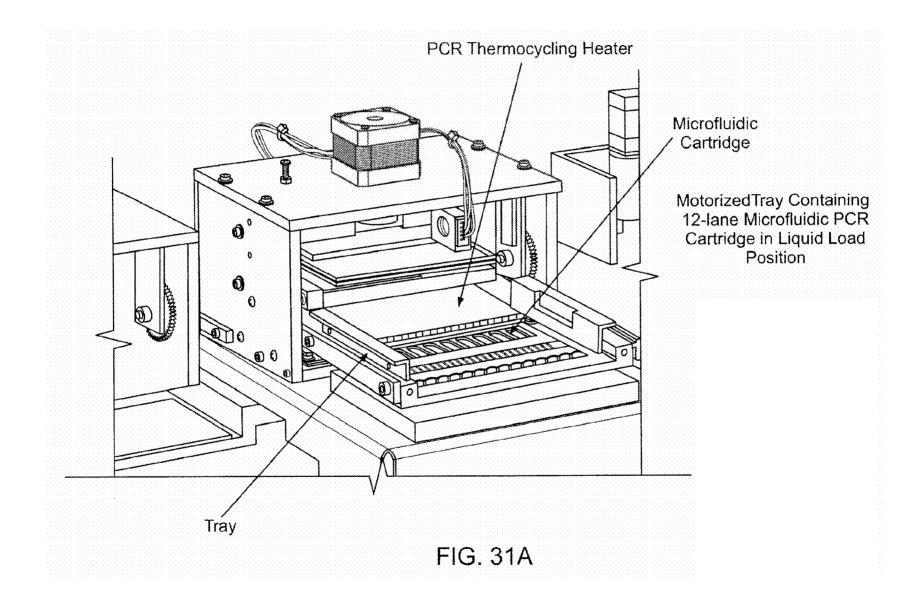
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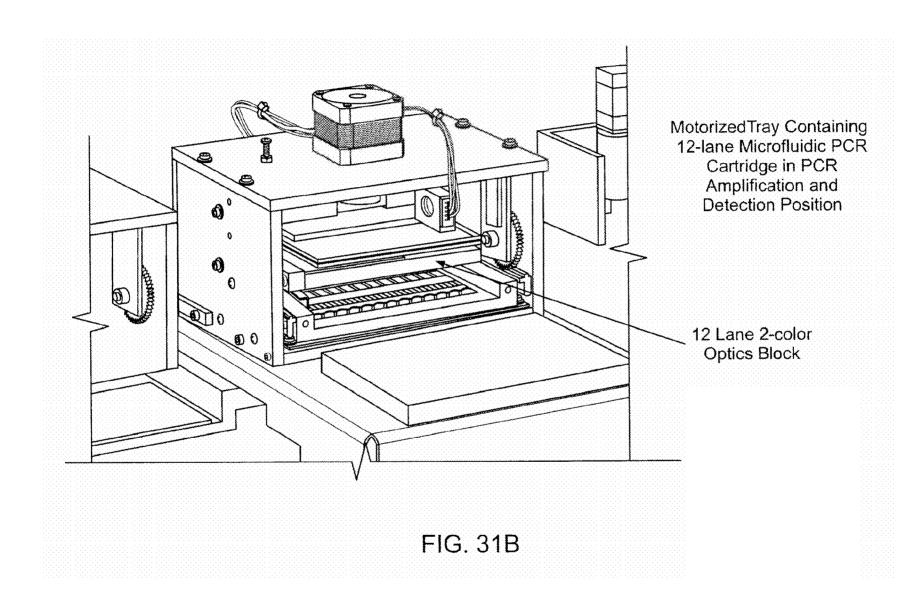
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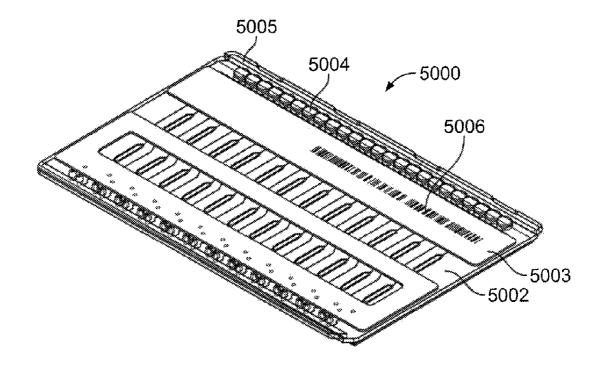


FIG. 32A

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FIG. 32B

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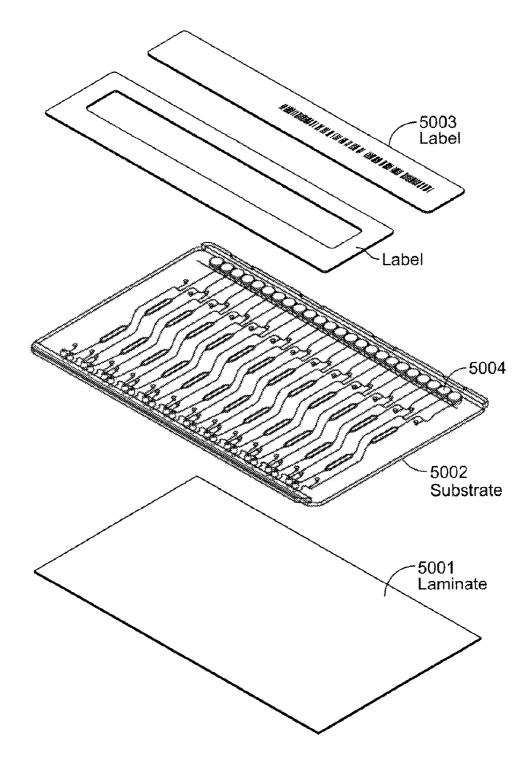


FIG. 32C

# 1 MICROFLUIDIC CARTRIDGE

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 12/239,537, filed on Sep. 26, 2008 (now U.S. Pat. No. 8,105,783), which is a continuation-in-part of U.S. patent application Ser. No. 12/173,023, filed on Jul. 14, 2008, which claims the benefit of priority to U.S. Provisional Patent Application No. 60/959,437, filed Jul. 13, 2007, all of which are incorporated herein by reference in their entirety. U.S. patent application Ser. No. 12/239,537, of which this application is a divisional, is also a continuation-in-part of U.S. patent 15 application Ser. No. 11/985,577, filed on Nov. 14, 2007 (now U.S. Pat. No. 7,998,708), which is incorporated herein by reference in its entirety. U.S. patent application Ser. No. 12/239,537, of which this application is a divisional, also claims benefit of priority to U.S. Design Patent Application 20 No. 29/308,920, filed Jul. 14, 2008 (now U.S. Design Pat. No. D621,060), which is incorporated herein by reference in its entirety.

### BACKGROUND OF THE INVENTION

### 1. Field of the Invention

The technology described herein generally relates to microfluidic cartridges. The technology more particularly relates to microfluidic cartridges that are configured to carry 30 out PCR on nucleotides of interest, particularly from multiple biological samples in parallel, within microfluidic channels, and permit detection of those nucleotides.

### 2. Description of the Related Art

The medical diagnostics industry is a critical element of today's healthcare infrastructure. At present, however, diagnostic analyses no matter how routine have become a bottleneck in patient care. There are several reasons for this. First, many diagnostic analyses can only be done with highly spe- $_{40}$ cialist equipment that is both expensive and only operable by trained clinicians. Such equipment is found in only a few locations—often just one in any given urban area. This means that most hospitals are required to send out samples for analyses to these locations, thereby incurring shipping costs and 45 transportation delays, and possibly even sample loss or mishandling. Second, the equipment in question is typically not available 'on-demand' but instead runs in batches, thereby delaying the processing time for many samples because they must wait for a machine to fill up before they can be run.

Understanding that sample flow breaks down into several key steps, it would be desirable to consider ways to automate as many of these as possible. For example, a biological sample, once extracted from a patient, must be put in a form suitable for a processing regime that typically involves using 55 PCR to amplify a vector of interest. Once amplified, the presence or absence of a nucleotide of interest from the sample needs to be determined unambiguously. Preparing samples for PCR is currently a time-consuming and labor intensive step, though not one requiring specialist skills, and 60 could usefully be automated. By contrast, steps such as PCR and nucleotide detection have customarily only been within the compass of specially trained individuals having access to specialist equipment.

There is a need for a method and apparatus of carrying out 65 sample preparation on samples in parallel, followed by PCR and detection on the prepared biological samples, and pref2

erably with high throughput. The PCR should be capable of being carried out by someone requiring minimal training, and should be fast.

The discussion of the background herein is included to explain the context of the inventions described herein. This is not to be taken as an admission that any of the material referred to was published, known, or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word "comprise" and variations thereof, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

### SUMMARY OF THE INVENTION

The present technology includes a microfluidic substrate, comprising: a plurality of sample lanes, wherein each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another: an inlet; a first valve and a second valve; a first channel leading from the inlet, via the first valve, to a reaction chamber; and a second channel leading from the reaction chamber, via the second valve, to a vent. The present technology further includes a 25 microfluidic cartridge comprising a microfluidic substrate having the aforementioned features.

The technology further includes a microfluidic cartridge, consisting of: a substrate having an upper side and an opposed lower side, wherein the substrate comprises a plurality of microfluidic networks arranged into a plurality of sample lanes; a laminate attached to the lower side; and a label, attached to the upper side. The cartridge can be further configured such that each lane of the plurality of lanes comprises a microfluidic network having, in fluid communication with one another: an inlet; a first valve and a second valve; a first channel leading from the inlet, via the first valve, to a reaction chamber; and a second channel leading from the reaction chamber, via the second valve, to a vent.

The technology further includes a method of carrying out PCR independently on a plurality of polynucleotide-containing samples, the method comprising: introducing the plurality of samples into a microfluidic cartridge, wherein the cartridge has a plurality of PCR reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another; moving the plurality of samples independently of one another into the respective plurality of PCR reaction chambers; isolating the plurality of PCR reaction chambers; and amplifying polynucleotides contained with the plurality of samples, by application of successive heating and cooling cycles independently to the PCR reaction chambers. In certain implementations, PCR is carried out simultaneously on two or more of the plurality of samples.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an exemplary microfluidic cartridge having a 3-layer construction.

FIGS. 2A-2E show various views of an embodiment of a microfluidic cartridge.

FIG. 3 shows a perspective view of an embodiment of a microfluidic cartridge.

FIGS. 4A-4D show various views of an embodiment of a microfluidic cartridge. FIG. 4E shows an overlay of a heater unit with the lanes of the cartridge.

FIGS. 5A-5B show diagrams of an exemplary microfluidic double valve. FIG. 5A additionally shows an exemplary valve in an open state, and the valve in a closed state.

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FIGS. 6A-6B show diagrams of exemplary single microfluidic valves.

FIG. 7 shows a hydrophobic vent suitable for use in a microfluidic network described elsewhere.

FIG. 8 shows an assembly process for a cartridge as further 5 described herein.

FIGS. 9A and 9B show exemplary deposition of wax droplets into microfluidic valves.

FIGS. 10A and 10B show an exemplary apparatus for carrying out wax deposition.

FIG. 11 shows a schematic of a diagnostic apparatus.

FIGS. 12A and 12B show exterior views of an exemplary apparatus.

FIG. 13 shows an exemplary interior view of an apparatus,  $_{15}$  illustrating a cartridge receiving bay.

FIG. 14 shows a microfluidic cartridge, and a cartridge tray.

FIG. **15** shows a cross-section of a pipetting head and a cartridge in position in a microfluidic apparatus.

FIG. **16** shows a view in cross-section of a microfluidic 20 cartridge situated in a receiving bay, adjacent a heater unit.

FIGS. 17A, 17B show a PCR reaction chamber and associated heaters.

FIG. 18 shows thermal images of heater circuitry in operation.

FIGS. 19A-19C shows various cut-away sections that can be used to improve cooling rates during PCR thermal cycling.

FIG. 20 shows a plot of temperature against time during a PCR process, as performed on a microfluidic cartridge as described herein.

FIGS. 21A-21C show views of an exemplary highly-multiplexed microfluidic cartridge, in plan (FIG. 21A), close-up of valves (FIG. 21B), and close up of inlets (FIG. 21C).

FIGS. **22**A and **22**B show various aspects of heater units associated with exemplary highly multiplexed microfluidic <sup>35</sup> cartridges.

FIGS. 23A-C show various aspects of a radially configured highly multiplexed microfluidic cartridge.

FIG. **24** shows a cartridge auto-loader in conjunction with an amplification-detection system.

FIG. 25 shows a perspective view, and a close-up view, of a cartridge stacker.

FIG. **26** shows a cartridge stacker in position to deliver a cartridge to an auto-loader.

FIG. 27 shows a cartridge loading system.

FIG. 28 shows a disposal unit for used cartridges.

FIG. **29** shows a cartridge stacker in full and empty configurations.

FIG. 30 shows a barcode reader positioned above a microfluidic cartridge.

FIGS. 31A and 31B show a thermocycling unit configured to accept a microfluidic cartridge.

FIG. 32A shows a plan view of a 24-lane microfluidic cartridge. FIG. 32B shows a perspective view of the cartridge of FIG. 32A. FIG. 32C shows an exploded view of the cartridge of FIG. 32A.

# DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present technology includes microfluidic cartridges that are configured to carry out PCR on multiple PCR-ready nucleic acid containing samples. The PCR-ready samples have typically been prepared by other devices, or components of an apparatus that accommodates the microfluidic cartridge during use, and introduced into the microfluidic cartridge prior to initiation of PCR.

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The microfluidic cartridges described herein are particularly effective for high throughput PCR applications because, for example, the small volumes of sample that are involved permit rapid heating and cooling cycles, and also mean that the PCR can be carried out on all of the samples in parallel in a relatively small space, thereby facilitating real-time analysis of multiple samples on a benchtop in a clinical setting.

Nucleic acid testing (NAT) as used herein is a general term that encompasses both DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) testing. Exemplary protocols that are specific to RNA and to DNA are described herein. It is to be understood that generalized descriptions where not specific to RNA or to DNA either apply to each equally or can be readily adapted to either with minor variations of the description herein as amenable to one of ordinary skill in the art. The terms nucleic acid and polynucleotide are used interchangeably herein.

Where used herein, the term "module" should be taken to mean an assembly of components, each of which may have separate, distinct and/or independent functions, but which are configured to operate together to produce a desired result or results. It is not required that every component within a module be directly connected or in direct communication with every other. Furthermore, connectivity amongst the various components may be achieved with the aid of a component, such as a processor, that is external to the module.

The microfluidic cartridges described herein are typically configured for use in a method and apparatus for carrying out sample preparation on biological samples in parallel, to create prepared (PCR-ready) samples, with PCR and detection on the prepared samples, and preferably with high throughput. Aspects of the operation of such apparatuses and their manner of communicating with microfluidic cartridges, are further described herein

Microfluidic Cartridge

One aspect of the present technology relates to a microfluidic cartridge that includes a first, second, and third layers that together define a plurality of microfluidic networks, each network having various components configured to carry out 40 PCR on a sample having one or more polynucleotides whose presence is to be determined. The cartridge includes one or more sample lanes in parallel, wherein each lane is independently associated with a given sample for simultaneous processing, and each lane contains an independently configured microfluidic network. The cartridge typically processes the one or more samples by increasing the concentration of (such as by amplification) one or more polynucleotides to be determined, as present in each of the samples. An exemplary cartridge having such a construction is shown in FIG. 1. Such a cartridge is simple to manufacture, and permits PCR to be carried out in a concentrated reaction volume (~4 µl) and enables rapid thermocycling, at ~20 seconds per cycle.

Although other layers may be found in cartridges having comparable performance and ease of manufacture, the cartridge herein includes embodiments having only three layers in their construction, as shown in the embodiment 3000 of FIG. 1, which has a substrate 3004, a laminate 3006, and a label 3002. In cartridge 3000, a microfluidic substrate 3004 has an upper side 3005 and, on an opposite side of the substrate (not visible in FIG. 1), a lower side 3007, wherein the substrate comprises a plurality of microfluidic networks, arranged into a corresponding plurality of sample lanes 3003; a laminate 3006 attached to the lower side 3007 of the substrate to seal the various components (such as valves) of the microfluidic networks, and to provide an effective thermal transfer layer between a dedicated heating element (further described herein) and components in the microfluidic net-

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works; and a label 3002, attached to the upper side 3005 of the substrate 3004, which also covers and seals holes that are used in the manufacturing process to load components such as valves of the microfluidic networks with thermally responsive materials.

Thus, embodiments of microfluidic cartridges herein include embodiments consisting of three layers, a substrate, a laminate, and a label, though other, additional, features other than layers may be consistent with such characterizations. Embodiments herein further include microfluidic cartridges 10 consisting essentially of three layers, a substrate, a laminate, and a label, though other, additional, features other than layers may be consistent with such characterizations. Furthermore, embodiments herein still further include microfluidic cartridges comprising three layers, a substrate, a laminate, 15 and a label.

The microfluidic substrate layer 3004 is typically injection molded out of a plastic, preferably a zeonor plastic (cyclic olefin polymer), and contains a number of microfluidic networks (not shown in FIG. 1), each having a PCR chamber, 20 channels, and valves on a first (e.g., lower) side (disposed towards the laminate), and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a second (e.g., upper) side (disposed toward the label). Typically, in a given cartridge, all of the microfluidic networks 25 together, including the PCR reactors, the inlet holes and the valves for isolating the PCR reaction chambers, are defined in a single substrate 3004. The substrate is made of a material that confers rigidity on the substrate (and hence the cartridge), and is impervious to air or liquid, so that entry or exit of air or 30 liquid during operation of the cartridge is only possible through the inlets or the various vent.

Channels of a microfluidic network in a lane of cartridge 3000 typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network 35 may have a width and/or a depth of less than 1 mm (e.g., about 750 microns or less, about 500 microns, or less, or about 250 microns or less).

The heat sealable laminate layer 3006 (typically between bottom surface of the microfluidic substrate using, for example, heat bonding, pressure bonding, or a combination thereof. The laminate layer 3006 may also be made from a material that has an adhesive coating on one side only, that side being the side that contacts the underside of the microf- 45 luidic substrate. This layer may be made from a single coated tape having a layer of Adhesive 420, made by 3M. Exemplary tapes include single-sided variants of double sided tapes having product nos. 9783, 9795, and 9795B, and available from 3M. The laminate layer is typically 50-200µ thick, for 50 example 125µ thick. Other acceptable layers may be made from adhesive tapes that utilize micro-capsule based adhe-

Typically, the label 3002 is made from polypropylene or other plastic with pressure sensitive adhesive (typically 55 between about 50 and 150 microns thick) and is configured to seal the wax loading holes of the valves in the substrate, trap air used for valve actuation, and serve as a location for operator markings. The label can be in two or more separate pieces, though it would be understood by one of ordinary skill in the 60 art that in many embodiments a single piece layer would be appropriate.

The label may be printed with various information, such as a manufacturer's logo, a part number, index numbers for each of the sample lanes, and an area 3012 where a user can make 65 annotations. In various embodiments, the label comprises a computer-readable or scannable portion 3008 that may con6

tain certain identifying indicia such as a lot number, expiry date, or a unique identifier. For example, the label can include a bar code, a radio frequency tag, or one or more computerreadable, or optically scannable, characters. The readable portion of the label can be positioned such that it can be read by a sample identification verifier as further described herein.

Microfluidic cartridge 3000 can be fabricated as desired, as further described herein.

A multi-lane cartridge, such as shown in FIG. 1, is configured to accept a number of samples, in particular embodiments 12 samples, through a number of inlets 3013 wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain one or more polynucleotides in a form suitable for amplification. The polynucleotides in question may be the same as, or different from one another, in different lanes of a cartridge. The multi-sample cartridge comprises at least a first microfluidic network and a second microfluidic network, adjacent to one another, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network accepts the first sample, and wherein the second microfluidic network accepts the second sample.

A sample lane, as further described herein, includes a network of microfluidic elements, controllable independently of those in another sample lane, by which a sample can be accepted and analyzed, according to methods described herein. A lane comprises at least a sample inlet, and a microfluidic component, as further described elsewhere herein. In some embodiments, each microfluidic network additionally comprises an overflow reservoir to contain surplus liquid dispensed into the cartridge.

A microfluidic network, in a microfluidic substrate of a cartridge, can include, in fluidic communication, one or more components selected from the group consisting of: gates, valves such as thermally actuated valves, channels, vents, and reaction chambers. Particular components of exemplary microfluidic networks are further described elsewhere herein.

The microfluidic network in each lane is typically configabout 100 and about 125 microns thick) can be attached to the 40 ured to carry out PCR on a PCR-ready sample, such as one containing nucleic acid (DNA or RNA) extracted from a raw biological sample using other aspects of the apparatus as further described herein. A PCR-ready sample is thus typically a mixture comprising the PCR reagent(s) and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide sample. For example, a PCRready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence. Examples of such reagents, and probes, are described elsewhere herein. A sample lane further typically includes a region 3014 of the substrate above a PCR reactor that permits a detector to monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotides.

> In various embodiments, a lane of a microfluidic cartridge can include a sample inlet port 3013, a first thermally actuated valve, a second thermally actuated valve, a PCR reaction chamber, and one or more channels connecting the inlet port to the PCR reaction chamber via the first valve, and one or more channels connecting the PCR reaction chamber to an exit vent via the second valve. The sample inlet valve can be configured to accept a quantity of sample at a pressure differential compared to ambient pressure of between about 100 to

5,000 Pa, such as can be delivered by an automated liquid dispenser (as described in U.S. patent application Ser. No. 12/212,403, filed Sep. 17, 2008, and incorporated herein by reference in its entirety). It should be noted that the lower the loading pressure, the higher the fill time for a aliquot of 5 reaction mix to fill the microfluidic network. Applying more pressure will reduce the fill time, but if the time for which the pressure is applied is not determined correctly, the sample could be blown out through the microfluidic cartridge (if an end hydrophobic vent is not present). Therefore the time for 10 which the pressure is applied should to be properly determined, such as by methods available to one of ordinary skill in the art, to prevent underfill or overfill. In general, the fill time is inversely proportional to the viscosity of the solution.

The sample inlets of adjacent lanes are reasonably spaced 15 apart from one another to prevent any contamination of one sample inlet from another sample when a user introduces a sample into any one cartridge. In some embodiments, the multi-sample cartridge is designed so that a spacing between recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge, as further described herein, is 8 mm. A spacing of 8 mm is convenient both for interfacing the cartridge with an automated pipetting apparatus, as described elsewhere 25 herein, as well as for interfacing with pipettes controlled manually that, e.g., transfer samples from PCR tubes to the cartridge. Thus, when used in conjunction with an automated sample loader having 4 heads, spaced equidistantly at 8 mm apart, the inlets having a 8 mm spacing can be loaded in three 30 batches of 4 inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from one side of the cartridge to the other.

One skilled in the art would recognize that other spacings, 35 such as 6 mm, 9 mm, 10 mm, and 12 mm, between centroids of sample inlets are consistent operation of the cartridge, as described elsewhere herein.

In some embodiments, the sample inlets are configured so as to prevent subsequent inadvertent introduction of sample 40 into a given lane after a sample has already been introduced into that lane.

The inlet holes 3013 can be manufactured frusto-conical in shape with an appropriate conical angle so that industrystandard pipette tips (2 µl, 20 µl, 200 µl, volumes, etc.) fit 45 snugly, entering from the widest point of the inlet. Thus, in certain embodiments, an inlet comprises an inverted frustoconical structure of at least 1 mm height, and having a diameter at its widest point that accepts entry of a pipette tip, of from 1-5 mm. The apparatus herein may be adapted to suit 50 other, later-arising, industry standards for pipette tips not otherwise described herein. Typically the volume of sample accepted via an inlet into a microfluidic network in a sample lane is from 1-20  $\mu$ l, and may be from 3-5  $\mu$ l. The inlet hole can be designed to fit a pipette tip snugly and to create a good 55 seal around the pipette tip, within the cone of the inlet hole. Once the pipette tip lands within the cone, the conical shape guides the pipette and mechanically seals the combination to provide error free dispensing or withdrawal of fluid into the cartridge. However, the cone is designed such that the sealing 60 is reversible because it is undesirable if the seal is so tight that the cartridge can be pulled away from its tray, or location in the receiving bay, when the pipette tips are lifted after the dispensing operations.

In some embodiments, the microfluidic cartridge further 65 comprises a registration member 3009 that ensures that the cartridge is received by a complementary diagnostic appara8

tus in a single orientation, for example, in a receiving bay of the apparatus. The registration member may be a simple cut-out from an edge or a corner of the cartridge (as shown in FIG. 1), or may be a series of notches, wedge or curvedshaped cutouts, or some other configuration of shapes that require a unique orientation of placement in the apparatus.

In some embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is customarily used in the art. Advantageously, then, the cartridge may be used with plate handlers used elsewhere in the

In some embodiments, the microfluidic cartridge comprises two or more positioning elements, or fiducials 3010, for use when filling the valves with thermally responsive material. The positioning elements may be located on the substrate, typically the upper face thereof. In the embodiment of FIG. 1, they are shown on diagonally opposed corners of the substrate but are not limited to such positions.

After PCR has been carried out on a sample, and presence the centroids of sample inlets is 8 mm, which is an industry- 20 or absence of a polynucleotide of interest has been determined, it is typical that the amplified sample remains on the cartridge and that the cartridge is either used again (if one or more lanes remain open), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the laminate of the cartridge, and recover an amount—typically about 1.5 microliter—of PCR product. The user may also place the individual PCR lane on a special narrow heated plate, maintained at a temperature to melt the wax in the valve, and then aspirate the reacted sample from the inlet hole of that PCR lane.

> The microfluidic cartridges may also be stackable, such as for easy storage or transport, or may be configured to be received by a loading device, as further described herein, that holds a plurality of cartridges in close proximity to one another, but without being in contact with one another. In order to accomplish either or both of these characteristics, the substrate may comprise two ledges or ridges, one of each of which is situated along each of two opposite edges of the cartridge, the ledges or ridges typically disposed on the upper side of the substrate. Thus, where a cartridge has a rectangular aspect (ignoring any registration member or mechanical key), the two ridges may be situated along the long sides, or along the short sides, of the cartridge. In the embodiment of FIG. 1, the two ridges 3011 are situated along the long sides of the cartridge. The ridges can be, e.g., 2 mm wide, and in general are sufficiently wide to accomplish stacking. The inlet holes are positioned sufficiently far from the ridges to prevent interference with liquid inputting operations.

> In various embodiments, during transport and storage, the microfluidic cartridge can be further surrounded by a sealed pouch to reduce effects of, e.g., water vapor. The microfluidic cartridge can be sealed in the pouch with an inert gas. The microfluidic cartridge can be disposable, such as intended for a single use.

> In use, a microfluidic cartridge, as described herein, is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, and PCR reactor) of the microfluidic networks. In some embodiments, the heat sources are controlled by an operating system, which operates the device during use. The operating system includes a processor (e.g., a computer) configured to actuate the heat sources according to a desired protocol. Processors configured to operate microfluidic devices are described in, e.g., U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference.

> The application of pressure (such as ~1 psi) to contact the cartridge to the heater of the instrument assists in achieving

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better thermal contact between the heater and the heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was partially filled with liquid and the entrapped air would be thermally expanded during thermocycling.

Three further exemplary embodiments of a microfluidic cartridge are shown in, respectively, FIGS. 2A-2E, 3, and 4.

FIG. 2A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. The cartridge may be referred to as a multi-lane PCR cartridge. FIGS. 2B, 2C, and 2D show respectively top plan, side, and bottom plan views of the cartridge of FIG. 2A. Visible in FIGS. 2A-2D are various representative components of cartridge 200. In particular, the embodiment of FIGS. 2A-2D contains twelve independent sample lanes 209 capable of independent (simultaneous or successive) processing of samples. Each lane has a dedicated pipette inlet 202. For example, sample inlet 202 is configured to accept a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown, wherein one inlet operates in conjunction with a single lane. Various compo- 20 nents of microfluidic circuitry in each lane are also visible and/or shown in FIG. 2E. For example, microvalves 204 and 206 are microfluidic components that are parts of microfluidic circuitry in a given lane. Also shown is an ultrafast PCR reactor 210, which, as further described herein, is a microfluidic channel that is long enough to permit PCR to occur in a sample. Typical dimensions of a PCR reactor are 150µ deep by 700μ wide, and a typical volume is ~1.6 μl. Above PCR reactor 210 is a window 212 that permits optical detection, such as detection of fluorescence from a fluorescent substance, such as a fluorogenic hybridization probe, in PCR reactor 210 when a detector is situated above window 212.

FIG. 3 shows schematically an aspect of the cartridge herein, that it is not limited to twelve lanes. The broken section in FIG. 3 illustrates that embodiments of the cartridge may differ in their aspect ratios so as to accommodate fewer than, or more than, twelve lanes. Numbers of lanes that may be found in embodiments of microfluidic cartridges consistent with the other description herein, include but are not limited to, 4, 6, 8, 10, 15, 16, 20, 24, 30, 32, 36, 40, and 48.

FIG. 4A shows a perspective view of an exemplary microf-luidic cartridge 400 having 12 lanes 409, though as with other cartridges herein, other numbers of lanes are consistent with the operation of this cartridge. The cartridge is particularly configured to accept samples from a manual pipetting device, rather than an automated pipette head. Tab 401 is optional, 45 and when present facilitates removal of the cartridge from a receiving bay into which it is loaded during use. Thus, a user grabs the tab 401, and pulls the cartridge out from a recessed location that hinders gripping the cartridge by is edges.

FIG. 4B shows a plan view of the underside of the embodiment of FIG. 4A, and depicts the microfluidic networks in each lane. FIG. 4C shows a perspective cutaway view of a portion of the top of the cartridge of FIG. 4A, showing various aspects of the microfluidic circuits. FIG. 4D shows the microfluidic circuit of a single lane of the cartridge of FIG. 55 4A.

### Microfluidic Networks

FIG. 2E (left panel) shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIG. 2A. FIG. 2E (right panel) shows how 60 the circuit is visible through the cartridge construction.

FIG. 4D shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. 4A and 4B.

Other configurations of microfluidic network, not shown in 65 FIGS. **2**E and **4**D, would be consistent with the function of the cartridges and apparatus described herein.

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The microfluidic networks of FIGS. 2E and 4D are now described, wherein it is apparent that like reference numerals refer to like elements. Throughout the operation of cartridge 200 the fluid is manipulated as a microdroplet (not shown in FIGS. 2E, 4D).

In sequence, sample-containing solution is introduced through liquid inlet 202 that communicates with inlet hole 203. The network optionally includes a bubble removal vent channel 208 into which sample flows and which permits adventitious air bubbles introduced into the sample during entry to escape. Typically, when using a robotic dispenser of liquid sample (e.g., as described in U.S. patent application Ser. No. 12/212,403, filed Sep. 17, 2008, and incorporated herein by reference), the volume is dispensed accurately enough that formation of bubbles is not a significant problem, and the presence of vent channel 208 is not necessary. Conversely, vent channel 208 finds most use when sample is introduced into the network via manual pipetting. After passing through the bubble vent, if present, sample continues along a channel 216; if there is no bubble removal vent, as in FIG. 2E, the sample solution flows directly from the inlet into channel 216.

Channel 216 is shown as kinked around the location of microvalve 206. Such a kink is not necessary, but merely convenient to ensure that the whole network occupies as small an area of the microfluidic substrate as is practical.

Typically, the microfluidic network is configured so that the time required for a microdroplet of sample to pass from the inlet to the second valve is less than 50% of the time required for the sample to travel up to the exit vent. Typically, the microfluidic network is designed to have an increased flow resistance downstream of the two valves without increasing the total volume of the microfluidic network in comparison to the amount required to fill from the first valve to the end vent of the network.

Valves 204 and 206 are shown in FIG. 4D as double-valves, having a source of thermally responsive material (also referred to as a temperature responsive substance) on either side of the channel where they are situated. However, as shown in FIG. 2E, valves 204 and 206 may either or both be single valves that have a source of thermally responsive material on only one side of the respective channels. Valves 204 and 206 are initially open, so that a microdroplet of samplecontaining fluid can be pumped into PCR reactor 210 from inlet hole 202. Upon initiating of processing of the sample, the detector present on top of the PCR reactor checks for the presence of liquid in the PCR reactor, and then, if liquid is present, communicates that fact to a processor controller that causes valves 204 and 206 to be closed to thereby isolate the PCR reaction mix from the channels on either side. Both valves 204 and 206 are closed prior to thermocycling to prevent any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor, during PCR. The use of microvalves configured such as valves 204 and 206 prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction.

The PCR reactor 210 is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein. Typically the PCR reactor has a volume of 3-5  $\mu$ l, and in particular embodiments has a volume of 4  $\mu$ l, or 4.5  $\mu$ l. For PCR reactors of such volumes, the input volume of fluid is typically from 4  $\mu$ l to 6  $\mu$ l. The inside walls of the channel in the PCR reactor are made very smooth and polished to a shiny finish (for example, using a polish such as SPI A1, SPI A2, SPI A3, SPI b1, or SPI B2) during manufacture. This is in order to minimize any microscopic air trapping in the surface of the PCR reactor, which would causing bubbling during the thermocycling steps. The presence of bubbles especially in the detec-

tion region of the PCR reactor might cause a false reading for, e.g., completion of PCR. Furthermore, the PCR reactor **210** is made shallow such that the temperature gradient across the depth of the channel is minimized. The region **212** of the substrate above PCR reactor **210** permits a detector to monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. The region **212** is made of thinner material than the rest of the cartridge so as to permit the PCR reactor to be more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denaturing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence.

In some embodiments, an end vent 214 prevents a user 15 from introducing any excess amount of liquid into the microfluidic cartridge, such as can easily be done during manual pipetting. As further described herein, an end vent is typically covered by a membrane. In other embodiments, an overflow chamber 229 plays a role of containing any sample from 20 spilling over to unintended parts of the cartridge. An overflow chamber is typically configured to capture 1-2 microliters of fluid. In still other embodiments, both an end vent and an overflow chamber 229 are present, and such that usually the end vent follows downstream of the overflow chamber. A user 25 may input sample volumes as small as an amount to fill from the bubble removal vent (where present) to the middle of the PCR reactor, or up to valve 204 or beyond valve 204. The end vent, overflow chamber, or combination thereof, serve to prevent excess fluid from flooding the microfluidic networks.

Also shown in FIG. 2E are residual portions 220 and 222, and 224 of structures that are removed from the cartridge substrate after manufacture. The presence of the structures during manufacture is to help with proper filling of the cartridge when molten plastic is flowed into the mold (mold flow). Items 220 and 224 are often called "dogbones" because of their shape. Still other shapes are of course possible and consistent with methods of manufacture and use, as described herein.

Also shown in FIG. 4D is a projection of a spacer element **219**. This item is also shown in FIG. 4C. It supports a label affixed over the vent such a way that it does not touch the vent.

In various embodiments, the microfluidic network can optionally include at least one reservoir configured to contain waste.

Table 1 outlines volumes, pumping pressures, and operation times associated with various components of a microfluidic network, as found in microfluidic cartridges described herein.

TABLE 1

Operation	Pumping Pressure	Displacement Volume	Time of Operation
Mixing displacements	~2 psi	10-25 µl	1-2 minutes
Moving valve wax plugs	~1-2 psi	<1 µl	5-15 seconds

Operation	Pump Used	Pump Design	Pump Actuation
Mixing displacements	Expancel Pump	Same as above	Same as above
Moving valve wax plugs	Thermopneunatic pump	1 μl of trapped air	Heat trapped air to ~70-90 C.

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Valves

A valve is a microfluidic component that has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Various valves find application in the microfluidic networks herein.

An exemplary double valve 3100 is shown in FIGS. 5A and 5B. A double valve has two channels 3101, 3103, one on either side of the channel 3102 whose flow it regulates, whereas a single valve, such as in FIGS. 6A, 6B, has just one channel, disposed on one side of the channel whose flow it regulates. Channels 3101, 3103 are referred to as loading channels because a thermally responsive substance (TRS) is loaded into such channels, for example via fluidly connected valve inlets 3105 and 3106.

Upon actuation, e.g., by application of heat, the valve transitions to a closed state that prevents material, such as a microdroplet of PCR-ready sample, from passing along the channel 3102 from one side of the valve to the other. For example, a valve includes one or more masses of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. Actuation of the valve involves transitioning the TRS from a temperature at or lower than the first temperature to a temperature that is at or higher than the second temperature.

A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage upon actuation. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. Generally, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less).

For each mass of TRS associated with a valve, a chamber is in gaseous communication with the mass. Upon heating gas (e.g., air) in the chamber(s) and heating the one or more masses of TRS to the second temperature, gas pressure within a chamber moves the corresponding mass into the channel obstructing material from passing therealong. Other valves of the microfluidic network have the same structure and operate in the same fashion as the valves described herein.

In order to make the valve sealing very robust and reliable, the flow channel 3102 at the valve junction is made narrow (150 μm wide and 150 μm deep or narrower) and the constricted portion of the channel is made at least 0.5 or 1 mm long such that the wax seals up a long narrow channel thereby reducing any leakage of fluid sample through the walls of the channel. In the case of a bad seal, there is leakage of fluid around the walls of the channel, past the wax. So the flow channel is narrowed as much as possible, and made longer, e.g., as long as ~1 mm.

The valve is operated by heating trapped pockets of air in

55 the valve inlets (also referred to as wax-loading ports), which
forces the TRS forwards in a manner so that it does not come
back to its original position. In this way, both air and TRS are
heated during operation of the valve.

FIGS. **6**A and **6**B show embodiments of single valves whose structures are consistent with use in the microfluidic networks described herein.

In various embodiments, the microfluidic network can include a single valve, also referred to as a bent valve, as shown in FIG. 6A (as a single valve) to reduce the footprint of the valve on the cartridge and hence reduce cost per part for manufacturing highly dense microfluidic substrates. In the valve of FIG. 6A, the loading hole for TRS is in the center of

13 the valve; the structures at either end are an inlet and an outlet and are shown for illustrative purposes only.

In various embodiments, the network can include a curved valve as shown in FIG. 6B, also as a single valve, in order to reduce the effective cross-section of the microvalve, also 5 enabling manufacture of cheaper dense microfluidic devices.

A hydrophobic vent (e.g., vent 3200 in FIG. 7) is a structure that permits gas to exit a channel while limiting (e.g., preventing) liquid from exiting the channel. Typically, hydro- 10 phobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from Osmonics) that defines a wall of the channel. As discussed herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microf- 15 luidic network. In embodiments such as shown in FIGS. 4A-4D, the membrane is present as a layer (such as of a oleophobic or hydrophobic material) positioned underneath one or more of the labels, and configured to cover the vent channels of microfluidic substrate. It can be applied using 20 heat bonding. A suitable material is a 0.2 to 1.0 micron poresize membrane of modified polytetrafluorethylene (typically between about 25 and about 100 microns thick).

The hydrophobic vents of the cartridge are preferably constructed so that the amount of air that escapes through them is 25 maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane of large surface area and a shallow cross section of the microchannel below the vent surface. An exemplary membrane is a 30 PTFE membrane having 0.22μ pore size. Other pore sizes are consistent with operation of the vents herein, and according to application.

Bubble removal hydrophobic vents typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least 35 about 7.5 mm) along a channel. The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel 3201 within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydropho- 40 bic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less). Bubble vents are optional in the microfluidic networks of the microfluidic cartridges described herein.

The depth of the channel within the hydrophobic vent is 45 typically about 75% or less (e.g., about 65% or less, about 60% or less) of than the depth of the channel upstream and downstream of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and 50 downstream of the hydrophobic vent is about 250 microns.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50% wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary 55 embodiment, the width of the channel within the hydrophobic vent is about 400 microns and the width of the channel upstream and downstream from the vent is about 250

The vent of FIG. 7 is a hydrophobic vent having a linear 60 channel 3201, connected via an inlet 3203 and an outlet 3204 to the rest of the microfluidic network. The right hand panel of FIG. 7 shows the vent in top plan view, and indicates where the membrane is situated.

Manufacturing Process for Cartridge

FIG. 8 shows a flow-chart 2800 depicting an assembly process for an exemplary cartridge as further described 14

herein. It would be understood by one of skill in the art, both that various steps may be performed in a different order from that set forth in FIG. 8, and additionally that any given step may be carried out by alternative methods to those set forth in the figure. It would also be understood that, where separate steps are illustrated for carrying out two or more functions, such functions may be performed synchronously and combined into single steps and still be consistent with the overall process described herein.

At 2802, a laminate layer is applied to a microfluidic substrate that has previously been engineered to have a plurality of microfluidic networks constructed in it; edges are trimmed from the laminate where they spill over the bounds of the substrate. The laminate seals in various components of the microfluidic networks.

At 2804, a thermally responsive substance such as wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate from the opposite side of the substrate to that sealed by the laminate. An exemplary process for carrying this out is further described herein.

At **2806**, the cartridge is inspected to ensure that wax from 2804 is loaded properly and that the laminate from 2802 adheres properly to the microfluidic substrate. If a substrate does not satisfy either or both of these tests, it is discarded. If substrates repeatedly fail either or both of these tests, then the wax dispensing, or laminate application steps, as applicable, are reviewed.

Optionally, at 2808, for cartridge embodiments that employ a hydrophobic vent, a hydrophobic vent membrane is applied to, and heat bonded to, the top of the microfluidic substrate over the wax-loaded valves, and on the opposite face of the substrate from the laminate. Edges of the membrane that are in excess of the boundary of the substrate are

Optionally, at 2810 if a vent membrane has been applied (2808), the assembly is inspected to ensure that the hydrophobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is discarded, and, in the case of repeated discard events, the foregoing process is reviewed.

At 2812, a label layer is applied to the top of the microfluidic substrate, situated to cover the valves. It would be understood that a multiple label parts, instead of a single layer, may be devised to fulfill both of these roles. In the embodiments in which a membrane has been affixed, the label(s) may comprise a portion that protects the vent membrane. The label typically has identifying indicia, such as a barcode, printed on it before it is applied to the top of the substrate.

At 2814 optionally, additional indicia are printed or applied to the label layer to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Typically one or more of the labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

At **2816**, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked and pack cartridges in groups, such as groups of 24 or 25, or groups of 10, or groups of 20, or groups of 50. Preferably the packaging is via an inert and/or moisture-free medium.

Wax Loading in Valves

FIGS. 9A and 9B show how a combination of controlled hot drop dispensing via a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The heated dispenser head (not shown in FIGS. 10A,

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10B) can be accurately positioned over an inlet hole of the microchannel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of  $\pm$ 20%. The inlet hole of the microchannel device is dimensioned in such a way that the droplet of 75 nl can be accurately shot to the bottom of the inlet hole using, for example, compressed air, or in a manner similar to an inkjet printing method, or by using a DJ-9000 manufactured by Asymtek, as further described herein.

The microchannel device is maintained at a temperature above the melting point of the wax thereby permitting the wax to stay in a molten state immediately after it is dispensed. After the drop falls to the bottom of the inlet hole, the molten wax is drawn into the narrow channel by capillary action. The volume of the narrow section is designed to be approximately equal to a maximum typical amount that is dispensed into the inlet hole.

The valve, shown in cross-section in FIGS. **9**A and **9**B, contains a step between the inlet and the loading channel. The step facilitates retention of the thermally responsive substance.

**Exemplary Wax-Deposition Process** 

Deposition of wax in valves of the microfluidic network, as at step **2804** (FIG. **8**) may be carried out with the exemplary equipment shown in FIGS. **10**A and **10**B. The DispenseJet <sup>25</sup> Series DJ-9000, FIGS. **10**A and **10**B (exploded view) is a non-contact dispenser that provides high-speed delivery and exceptional volumetric control for various fluids, including surface mount adhesive, underfill, encapsulants, conformal coating, UV adhesives, and silver epoxy.

The DJ-9000 jets in tight spaces as small as 200 micrometers and creates fillet wet-out widths as small as 300 micrometers on the dispensed side of a substrate such as a die. It dispenses fluid either as discrete dots or a rapid succession of dots to form a 100-micron (4 mil) diameter stream of fluid from the nozzle. It is fully compatible with other commercially available systems such as the Asymtek Century C-718/C-720, Millennium M-2000, and Axiom X-1000 Series Dispensing Systems.

A DJ-9000 is manufactured by Asymtek under manufacturing quality control standards aim to provide precise and reliable performance. Representative specifications of the apparatus are as follows.

Characteristic	Specification
Size	Width: 35 mm
	Height: 110 mm
	Depth: 100 mm
Weight	400 grams - dry
Feed Tube Assembly	Nylon - Fitting
	Polyurethane - Tube
Fluid Chamber	Type 303 Stainless Steel
Seat and Nozzle	300/400 Series S/S, Carbide
Needle Assembly	52100 Bearing Steel - Shaft
	Hard Chrome Plate
	Carbide - Tip
Fluid Seal	PEEK/Stainless Steel
Fluid Chamber 0-Ring	Ethylene Propylene
Jet Body	6061-T6 Aluminum
	Nickel Plated
Needle Assembly Bearings	PEEK
Thermal Control Body	6061-T6 Aluminum
	Nickel Plated
Reservoir Holder	Acetyl
Reservoir Size	5, 10, or 30 cc (0.17, 0.34, or 1.0 oz)
Feed Tube Assembly Fitting	Female Luer per ANSI/HIMA
	MD70.1-1983
Maximum Cycle Frequency	200 Hz.

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	Characteristic	Specification
5	Minimum Valve Air Pressure Operating Noise Level Solenoid Thermal Control Heater Thermal Control RTD Maximum Heater Set Point	5.5 bar (80 psi) 70 db* 24 VDC, 12.7 Watts 24 VDC, 14.7 Watts, 40 ohms 100 ohm, platinum 80 C.

\*At Maximum Cycle Rate

The DJ-9000 has a normally closed, air-actuated, spring-return mechanism, which uses momentum transfer principles to expel precise volumes of material. Pressurized air is regulated by a high-speed solenoid to retract a needle assembly 2903 from seat 2901. Fluid, fed into the fluid chamber 2902 from the fluid reservoir 2904, flows over the seat. When the air is exhausted, the needle travels rapidly to the closed position, displacing fluid through the seat and nozzle in the form of a droplet. Multiple droplets fired in succession can be used to form larger dispense volumes and lines when combined with the motion of a dispenser robot that controls motion and positioning of the dispense head.

The equipment has various adjustable features: The following features affect performance of the DJ-9000 and are typically adjusted to fit specific process conditions.

Fluid Pressure should be set so that fluid fills to the seat, but should not be influential in pushing the fluid through the seat and nozzle. In general, higher fluid pressure results in a larger volume of material jetted.

The stroke adjustment knob 2905 controls the travel distance of the needle assembly 2903. For example, the control can be turned counterclockwise to increase needle assembly travel, or turned clockwise to decrease travel. An increase of travel distance will often result in a larger volume of material jetted.

The solenoid valve 2906 controls the valve operation. When energized, it allows air in the jet air chamber to compress a spring 2907 and thereby raise the needle assembly. When de-energized, the air is released and the spring forces the piston down so that the needle tip contacts the seat.

The seat and nozzle geometry are typically the main factors controlling dispensed material volume. The seat and nozzle size are determined based on the application and fluid properties. Other parameters are adjusted in accordance with seat and nozzle choices. Available seat and nozzle sizes are listed in the table hereinbelow.

Fluid temperature often influences fluid viscosity and flow characteristics. The DJ-9000 is equipped with a thermal control assembly **2908** that assures a constant fluid temperature.

In addition to the DJ-9000 hardware configuration and settings, Dot and Line Parameters can be set in a software program (for example, one referred to as FmNT) to control the size and quality of dots and lines dispensed.

Packaging

The microfluidic cartridge described herein may be provided in a convenient package containing multiple cartridges, and made easily accessible, but suitably protected during carriage. In some implementations, the packaging comprises a plastic pouch to protect the cartridges from moisture, but this is not a requirement. The packaging material in general comprise one or more of paper, foam, and cardboard. In some implementations, the packaging facilitates lifting a stack, such as 24 cartridges, for easy transfer into a cartridge autoloader, as described elsewhere herein.

Apparatus Overview

The microfluidic cartridges as described herein find application to analyzing any nucleic acid containing sample for any purpose, including, but not limited to, genetic testing, and clinical testing for various infectious diseases in humans, and food testing, for example of agricultural products.

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The microfluidic cartridges herein are configured for use with a diagnostic apparatus. Such an apparatus is described in U.S. patent application Ser. No. 12/173,023, filed Jul. 14, 2008, incorporated herein by reference. Salient features of such an apparatus are now described herein. However, it would be understood that cartridges such as those herein could be configured for use with other types of apparatus not otherwise described herein, for example an apparatus that is configured to just perform PCR on samples that had previously been brought into PCR-ready form by some other apparatus, or manually.

The apparatus of FIG. 11 is configured to act on a disposable microfluidic cartridge containing multiple sample lanes in parallel, and comprises a reusable instrument platform that can actuate on-cartridge operations, can detect and analyze the products of the PCR amplification in each of the lanes separately, in all simultaneously, or in groups simultaneously, and, optionally, can display the results on a graphical user interface

A schematic overview of an apparatus 981 for carrying out automated sample preparation and diagnostic testing on multiple samples in parallel, according to steps exemplified elsewhere herein, is shown in FIG. 11. The geometric arrangement of the components of system 981 is exemplary and not intended to be limiting. In overview, multiple samples are introduced into the apparatus and individually placed into a form suitable for applying PCR to amplify one or nucleotides contained therein. The PCR-ready samples are transferred to a microfluidic cartridge that is acted on by the apparatus and caused to amplify nucleotides in the samples by carrying out PCR thereon, whilst in the cartridge. Results of the amplifications can be detected while the amplified nucleotides are in the cartridge.

A processor 980, such as a microprocessor, is configured to control functions of various components of the system as shown, and is thereby in communication with each such component requiring control, for example via a bus. It is to be understood that many such control functions can optionally be carried out manually, and not under control of the processor. Furthermore, the order in which the various functions are described, in the following, is not limiting upon the order in which the processor executes instructions when the apparatus is operating. A suitable processor 980 can be designed and manufactured according to, respectively, design principles and semiconductor processing methods known in the art.

Processor 980 can be configured to accept user instructions from an input device 984, where such instructions may include instructions to start analyzing the sample, and choices of operating conditions. Processor 980 can be also configured to communicate with a display 982, so that, for example, information about an analysis is transmitted to the display and thereby communicated to a user of the system. Such information includes but is not limited to one or more of: the current status of the apparatus; progress of PCR thermocycling; and a warning message in case of malfunction of either system or cartridge. Additionally, processor 980 may transmit one or more questions to be displayed on display 982 that prompt a user to provide input in response thereto. Thus, in certain embodiments, input 984 and display 982 are integrated with one another.

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Processor 980 can be optionally further configured to transmit results of an analysis to an output device 986 such as a printer, a visual display such as display 982 or a second display, a display that utilizes a holographic projection, or a speaker, or a combination thereof. Processor 980 can be still further optionally connected via a communication interface such as a network interface to a computer network 988.

Processor 980 can be further configured to control various aspects of sample preparation and diagnosis, as follows in overview. In FIG. 1, the apparatus 981 is configured to operate in conjunction with a complementary rack 970. Apparatus 981 may be capable of receiving multiple racks, such as 1, 2, 3, 4, or 6 racks.

Embodiments of rack 970 are further described in U.S. patent application Ser. No. 12/173,023, filed by Express Mail on Jul. 14, 2008 (and entitled "Integrated Apparatus for Performing Nucleic Acid Extraction and Diagnostic Testing on Multiple Biological Samples", in the name of Williams, et al.), and Ser. No. 12/178,584, filed on Jul. 23, 2008, and entitled "Rack For Sample Tubes And Reagent Holders", in the name of Duffy, et al., both of which are incorporated herein by reference in their entireties. A rack 970 is itself configured to receive a number of biological samples 996, such as nucleic-acid containing samples, in a form suitable for work-up and subsequent diagnostic analysis, and a number of holders 972—as further described herein, such as in connection with FIG. 2, that are equipped with various reagents, pipette tips and receptacles. The rack is configured so that, during sample work-up, samples are processed in the respective holders, the processing including being subjected, individually, to heating and cooling via heater assembly 977.

The heating functions of the heater assembly 977 can be controlled by the processor 980. Heater assembly 977 operates in conjunction with a separator 978, such as a magnetic separator, that also can be controlled by processor 980 to move into and out of close proximity to one or more processing chambers associated with the holders 972, wherein particles such as magnetic particles are present. Assembly 977 and separator 978 are further described in U.S. patent application Ser. No. 12/178,586, filed on Jul. 23, 2008, and entitled "Integrated Heater and Magnetic Separator", in the name of Handique, which is incorporated herein by reference in its entirety.

Processor 980 can be configured to receive data about a sample to be analyzed, e.g., from a sample reader 990, which may be a barcode reader, an optical character reader, or an RFID scanner (radio frequency tag reader). Thus, sample reader 990 is configured to transmit identifying indicia about the sample, and in some instances the holder, to processor 980. In some embodiments, the sample reader is movable from one sample position to another. In some embodiments a sample reader is attached to the liquid dispenser 976 and can thereby read indicia about a sample above which the liquid dispenser is situated. In other embodiments the sample reader is not attached to the liquid dispenser and is independently movable, under control of the processor.

Liquid dispenser 976, which similarly can be controlled by processor 980 and is further described herein, is configured to carry out various suck and dispense operations on respective samples in rack 970, and fluids and reagents in the holders 972, to achieve extraction of nucleic acid from the samples. Liquid dispenser 976 can carry out such operations on multiple holders simultaneously, and is further described herein.

In the embodiment of a diagnostic apparatus shown in FIG. 1, a cartridge 994 is received in bay 992. The receiving bay is in communication with a heater 998 that itself can be controlled by processor 980 in such a way that specific regions of the cartridge 994 are heated at specific times during analysis. Liquid dispenser 976 is configured to take aliquots of fluid

containing nucleic acid extracted from one or more samples and direct them to one or more respective inlets in cartridge **994**. Cartridge **994** is configured to amplify, such as by providing chambers for carrying out PCR on, the respective nucleic acids. The processor is also configured to control and 5 receive data from a detector **999** that receives an indication of a diagnosis from the cartridge **994**. The diagnosis can be transmitted to the output device **986** and/or the display **982**, as described hereinabove.

Embodiments of the apparatus shown in outline in FIG. 1, as with other exemplary embodiments described herein, are advantageous because they do not require locations within the apparatus suitably configured for storage of reagents. Therefore, the apparatus in FIG. 1 is self-contained and operates in conjunction with holders 972 and cartridges 994, wherein the 15 holders are pre-packaged with reagents, such as in locations within it dedicated to reagent storage, and wherein the cartridges are supplied with separately packaged PCR reagents appropriate for mixing with PCR-ready sample, prior to introduction into the cartridge.

The apparatus of FIG. 1 may be configured to carry out operation in a single location, such as a laboratory setting, or may be portable so that they can accompany, e.g., a physician, or other healthcare professional, who may visit patients at different locations. The apparatus is typically provided with a 25 power-cord so that it can accept AC power from a mains supply or generator. The apparatus may also be configured to operate by using one or more batteries and therefore is also typically equipped with a battery recharging system, and various warning devices that alert a user if battery power is 30 becoming too low to reliably initiate or complete a diagnostic analysis.

The apparatus of FIG. 1 may further be configured, in other embodiments, for multiplexed sample analysis and/or analysis of multiple batches of samples, where, e.g., a single rack 35 holds a single batch of samples. Each component shown in FIG. 1 may therefore be independently present as many times as there are batches of samples (or some fraction thereof), though the multiple instances of the various components may be configured in a common housing.

In various embodiments, preparation of a PCR-ready sample for use in subsequent diagnosis using the apparatus as further described herein can include one or more of the following steps: contacting a neutralized polynucleotide sample with a PCR reagent mixture comprising a polymerase 45 enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid); in some embodiments, the PCR reagent mixture can be in the form of one or more 50 lyophilized pellets, as stored in a receptacle on a holder, and the method can further include reconstituting the PCR pellet with liquid to create a PCR reagent mixture solution.

The apparatus herein can be configured to run on a laboratory benchtop, or similar environment, and can test approximately 45 samples per hour when run continuously throughout a normal working day. Results from individual raw samples are typically available in less than 1 hour.

FIGS. 12A and 12B show views of an exemplary diagnostic apparatus 3000 incorporating various elements of FIG. 11. 60 Shown in FIG. 12A, a front plan view of apparatus 3000 has a hinged cover 3010, shown in a closed position, bearing an optional clear window 3012 (that provides a user with an at-a-glance indication of the apparatus' status) and a handle 3014 that facilitates opening and closing of the cover.

Shown in FIG. 12B is a front plan view of apparatus 3000 with cover 3010 moved to an open position revealing certain

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elements of the interior 3020 of the apparatus. Aspects of the interior of the apparatus that are visible in the view of FIG. 12B include: two removable racks 970, each bearing 12 holders 972, a liquid dispenser 976, mounted on a gantry 2102, and a receiving bay 992 for holding a microfluidic cartridge, as further described herein.

Various aspects of an apparatus configured for use with a microfluidic cartridge are now further described. Cartridge Receiving Bay

In some embodiments, an apparatus includes a bay configured to selectively receive a microfluidic cartridge, at least one heat source thermally coupled to the bay and coupled to a processor as further described herein, such that the heat source is configured to heat individual sample lanes in the cartridge, and the processor is configured to control application of heat to the individual sample lanes, separately, in all simultaneously, or in groups simultaneously.

In various embodiments, the microfluidic networks in the cartridge can be configured to couple heat from an external 20 heat source, such as in the receiving bay, to a sample mixture comprising PCR reagent and PCR-ready, such as neutralized, polynucleotide sample and caused to undergo thermal cycling conditions suitable for creating PCR amplicons from the PCR-ready sample.

FIG. 13 shows an interior view of an exemplary apparatus, showing a rack holding a number of sample tubes and reagent holders, and a cartridge 994 situated in receiving bay 992.

FIG. 14 shows a perspective view of an exemplary cartridge 200 that contains multiple sample lanes, and a removeable receptacle shown as tray 110 that, optionally, can accommodate cartridge 200 prior to insertion of the cartridge in a receiving bay in an apparatus. Tray 110 makes it easier for the user to place the cartridge into a receiving bay such as in a diagnostic apparatus. Alignment of the cartridge into a removeable receptacle such as tray 110 is often easier than trying to seat the cartridge directly into a recessed area. Thus, tray 110 typically has a registration member that engages with a registration member, such as a notch or corner-cut-out, on the cartridge, and tray 110 also has raised portions that can be held in a user's hand during placement and removal of the tray+cartridge into a receiving bay. Tray 110 is also typically configured so that it holds the cartridge but structural elements of the tray do not interpose themselves between the cartridge and, e.g., a detector or a heating element, when the cartridge is in use.

The bay can be a portion of the apparatus that is configured to selectively receive the microfluidic cartridge. For example, the bay and the microfluidic cartridge can be complementary in shape so that the microfluidic cartridge is selectively received in, e.g., a single orientation. For example, the microfluidic cartridge can have a registration member that fits into a complementary feature of the bay. The registration member can be, for example, a cut-out on an edge of the cartridge, such as a corner that is cut-off, or one or more notches that are made on one or more of the sides. By selectively receiving the cartridge, the bay can help a user to place the cartridge so that the apparatus can properly operate on the cartridge. In this way, error-free alignment of cartridges can be achieved. Moreover, the cartridge can be designed to be slightly smaller than the receiving bay by approximately 200-300 micron for easy placement and removal of the cartridge. The apparatus can further include a sensor configured to sense whether the microfluidic cartridge is selectively received.

The bay can also be configured so that various components of the apparatus that can operate on the microfluidic cartridge (heat sources, detectors, force members, and the like) are

positioned to properly operate on the microfluidic cartridge while the cartridge is received in the bay. For example, a contact heat source can be positioned in the bay such that it can be thermally coupled to a distinct location at a microflu-

idic cartridge that is selectively received in the receiving bay. 5

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Alternatively, in connection with alignment of microheaters in the heater module with corresponding heat-requiring microcomponents (such as valves, pumps, gates, reaction chambers, etc.), the microheaters can be designed to be slightly bigger than the heat requiring microfluidic components so that even though the cartridge may be off-centered from the heater, the individual microfluidic components can still function effectively.

The heat source can be, for example, a heat source such as a resistive heater or network of resistive heaters, a reversible 15 heat source such as a liquid-filled heat transfer circuit or a thermoelectric element, a radiative heat source such as a xenon lamp, and the like.

In preferred embodiments, the at least one heat source can be a contact heat source selected from a resistive heater (or 20 network thereof), a radiator, a fluidic heat exchanger and a Peltier device. The contact heat source can be configured at the receiving bay to be thermally coupled to one or more distinct locations of a microfluidic cartridge received in the bay, whereby the distinct locations are selectively heated. At 25 least one additional contact heat source can be included, wherein the contact heat sources are each configured at the bay to be independently thermally coupled to a different distinct location in a microfluidic cartridge received in the bay, whereby the distinct locations are independently heated. 30 The contact heat source can be configured to be in direct physical contact with a distinct location of a microfluidic cartridge received in the bay. In various embodiments, each contact source heater can be configured to heat a distinct location having an average diameter in 2 dimensions from 35 about 1 millimeter (mm) to about 15 mm (typically about 1 mm to about 10 mm), or a distinct location having a surface area of between about 1 mm<sup>2</sup> about 225 mm<sup>2</sup> (typically between about 1 mm<sup>2</sup> and about 100 mm<sup>2</sup>, or in some embodiments between about 5 mm<sup>2</sup> and about 50 mm<sup>2</sup>).

In various embodiments, at least one heat source can be a radiative heat source configured to direct heat to a distinct location of a microfluidic cartridge received in the receiving bay. The bottom surface of the cartridge typically mates with the heating surface to form a snug fit.

In various embodiments, the apparatus includes one or more force members that are configured to apply force to thermally couple the at least one heat source to at least a portion of the microfluidic cartridge received in the bay. The one or more force members can be configured to operate a 50 mechanical member at the microfluidic cartridge. Typically the mechanical member at the cartridge is held in place by a motor that applies pressure to the member. In some embodiments, a force member can be manually operated. At least one force member can be mechanically coupled to a lid at the 55 receiving bay, whereby operation of the lid operates the force member. In various embodiments, the force applied by the motor or the one or more force members can result in an average pressure at an interface between a portion of the receiving bay and a portion of the microfluidic cartridge of 60 about 1 psi. The application of force is important to ensure consistent thermal contact between the heater wafer and the PCR reactor and microvalves in the microfluidic cartridge.

In various embodiments, the apparatus can further include a lid at the receiving bay, the lid being operable to at least 65 partially exclude ambient light from the bay. The lid can be, for example, a sliding lid but is more typically a hinged, 22

sprung, or motor operated lid that comes down on the cartridge from above. The lid can include the optical detector. A major face of the lid at the bay can vary from planarity by less than about 100 micrometers, for example, less than about 25 micrometers. The lid can be configured to be removable from the apparatus. The lid can include a latching member that ensures that the lid is securely closed before amplification reactions are applied to the samples in the cartridge.

FIG. 15 shows a schematic cross-sectional view of a part of an apparatus as described herein, showing input of sample into an inlet 202 of cartridge 200 via a pipette tip 10 (such as a disposable pipette) attached to an automated dispensing head. Although not shown, there are as many inlets 202 as samples to be input into cartridge 200. Inlet 202 is preferably configured to receive a pipette or the bottom end of a PCR tube and thereby accept sample for analysis with minimum waste, and with minimum introduction of air. Cartridge 200 is disposed on top of and in contact with a heater substrate 400. Read head 300 is positioned above cartridge 200 and a cover for optics 310 restricts the amount of ambient light that can be detected by the read head.

The detector can be, for example, an optical detector, as further described herein. For example, the detector can include a light source that selectively emits light in an absorption band of a fluorescent dye, and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. Alternatively, for example, the optical detector can include a bandpass-filtered diode that selectively emits light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof; or for example, the optical detector can be configured to independently detect a plurality of fluorescent 40 dyes at a plurality of different locations on a microfluidic cartridge, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof in a different sample.

Advantageously, in some embodiments the receiving bay design allows easy placement of the microfluidic cartridge, such as by a user, or an auto-loading device as further described herein. Such a design also accommodates multiple sample pipetting of liquid using a robotic dispenser, and optical detection in situ. Furthermore, it is typically easier to move a cartridge and heater in and out of position than a detector.

Heater Configurations to Ensure Uniform Heating of a Region

Another aspect of the apparatus described herein relates to uniform control of the heating of a region of a microfluidic network, the region including to one or more microfluidic components. In an exemplary embodiment, multiple heaters can be configured to simultaneously and uniformly heat a region, such as the PCR reaction zone, of a microfluidic network in the microfluidic cartridge.

In preferred embodiments, a microfluidic cartridge having one or more microfluidic networks, each comprising one or more microfluidic components, is brought into contact with a heat source, within a suitably configured apparatus. The heat source is configured so that particular heating elements are situated to heat specific components of the microfluidic networks of the cartridge.

FIG. 16 shows a cross-sectional view of an exemplary microfluidic cartridge, situated in a receiving bay, to show relative location of PCR chamber 901 in relation to the heaters when the cartridge is placed in the instrument. PCR chamber 901 is shown in a substrate layer 907 of the cartridge. A

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ber 901 is shown in a substrate layer 907 of the cartridge. A 5 laminate layer 905 of the cartridge is directly under the PCR chamber 901.

Two long heaters **909** and **911** that run alongside (when viewed from above) PCR chamber **901** are situated in a substrate layer **913** of the receiving bay, directly under and in contact with the laminate layer of the cartridge. The heaters are photolithographically defined and etched metal layers of gold (typically about 3,000 .ANG. thick). Layers of 400 .ANG. of TiW are deposited on top and bottom of the gold layer to serve as an adhesion layer. The substrate used can be 15 a glass, fused silica, or quartz wafer having a thickness of 0.2-1 mm, such as 0.4 mm, 0.5 mm or 0.7 mm, or 1 mm. A thin electrically-insulative layer of 2 µm silicon oxide serves as an insulative layer on top of the metal layer. Additional thin

also be deposited on top of the silicon oxide surface. Referring to FIGS. 17A and 17B, the PCR reaction chamber 1001 is configured with a long side and a short side, each with an associated heating element. The apparatus therefore preferably includes four heaters disposed along the sides of, 25 and configured to heat, the PCR reaction zone: long top heater 1005, long bottom heater 1003, short left heater 1007, and short right heater 1009. The small gap between long top heater 1005 and long bottom heater 1003 results in a negligible temperature gradient (less than 1° C. across the width of 30 the PCR channel at any point along the length of the PCR reaction zone) and therefore an effectively uniform temperature throughout the PCR reaction zone. The heaters on the short edges of the PCR reactor provide heat to counteract the gradient created by the two long heaters from the center of the 35 reactor to the edge of the reactor.

It would be understood by one of ordinary skill in the art that still other configurations of one or more heater(s) situated about a PCR reaction zone are consistent with the methods and apparatus described herein. For example, a 'long' side of 40 the reaction zone can be configured to be heated by two or more heaters. Specific orientations and configurations of heaters are used to create uniform zones of heating even on substrates having poor thermal conductivity because the poor thermal conductivity of glass, or quartz, or fused silica substrates is utilized to help in the independent operation of various microfluidic components such as valves and independent operation of the various PCR lanes.

In preferred embodiments, each heater has an associated temperature sensor. In the embodiment of FIG. 17A, a single 50 temperature sensor 1011 is used for both long heaters. A temperature sensor 1013 for short left heater, and a temperature sensor 1015 for short right heater are also shown. The temperature sensor in the middle of the reactor is used to provide feedback and control the amount of power supplied to 55 the two long heaters, whereas each of the short heaters has a dedicated temperature sensor placed adjacent to it in order to control it. As further described herein, temperature sensors are preferably configured to transmit information about temperature in their vicinity to the processor at such times as the 60 heaters are not receiving current that causes them to heat. This can be achieved with appropriate control of current cycles.

In order to reduce the number of sensor or heater elements required to control a PCR heater, the heaters may be used to sense as well as heat, and thereby obviate the need to have a 65 separate dedicated sensor for each heater. In another embodiment, each of the four heaters may be designed to have an

appropriate wattage, and connect the four heaters in series or in parallel to reduce the number of electronically-controllable

electronics.

FIG. 17B shows expanded views of heaters and temperature sensors used in conjunction with a PCR reaction zone of FIG. 17A. Temperature sensors 1001 and 1013 are designed to have a room temperature resistance of approximately 200-300 ohms. This value of resistance is determined by controlling the thickness of the metal layer deposited (e.g., a sandwich of 400 .ANG. TiW/3000 .ANG. Au/400 .ANG. TiW), and etching the winding metal line to have a width of approximately 10-25  $\mu m$  and 20-40 mm length. The use of metal in this layer gives it a temperature coefficient of resistivity of the order of 0.5-20° C./ohms, preferably in the range of 1.5-3° C./ohms. Measuring the resistance at higher temperatures will enable determination of the exact temperature of the location of these sensors.

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elements from 4 to just 1, thereby reducing the burden on the

insulative layer on top of the metal layer. Additional thin electrically insulative layers such as 2-4 µm of parylene may 20 for a single PCR reaction chamber, can be applied to a multi-laso be deposited on top of the silicon oxide surface.

Referring to FIGS. 17A and 17B, the PCR reaction chamber.

Each heater can be independently controlled by a processor and/or control circuitry used in conjunction with the apparatus described herein. FIG. 18 shows thermal images, from the top surface of a microfluidic cartridge having heaters configured as in FIGS. 17A and 17B, when each heater in turn is activated, as follows: (A): Long Top only; (B) Long Bottom only; (C) Short Left only; (D) Short Right only; and (E) All Four Heaters on. Panel (F) shows a view of the reaction zone and heaters on the same scale as the other image panels in FIG. 18. Also shown in the figure is a temperature bar. Use of Cutaways in Cartridge and Heater Substrates to Improve Rate of Cooling During PCR Cycling

During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved efficiency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the cartridge and to the heating substrate, as shown in FIGS. 19A-19C.

One way to achieve rapid cooling is to cutaway portions of the microfluidic cartridge substrate, as shown in FIG. 19A. The upper panel of FIG. 19A is a cross-section of an exemplary microfluidic cartridge and heater unit taken along the dashed line A-A' as marked on the lower panel of FIG. 19A. PCR reaction chamber 901, and representative heaters 1003 are shown. Also shown are two cutaway portions, one of which labeled 1201, that are situated alongside the heaters that are situated along the long side of the PCR reaction zone. Cutaway portions such as 1201 reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction zone. A further cutout portion 919 in the cartridge directly above the PCR chamber serves to both reduce thermal mass and background fluorescence. The latter is useful because, since cutout 919 is situated directly above the PCR chamber, the amount of material between the chamber and a fluorescence detector is reduced. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology.

Another way to achieve rapid cooling is to cutaway portions of the heater substrate, as shown in FIG. 19B. The lower panel of FIG. 19B is a cross-section of an exemplary microfluidic cartridge and heater substrate 921 taken along the dashed line A-A' as marked on the upper panel of FIG. 19B. PCR reaction chamber 901 situated in substrate 923 of the

cartridge, and representative heaters 1003 are shown. Laminate layer 921 of the cartridge is situated on top of heater substrate 921. The cartridge has a cutout 919 above the PCR chamber, as described in connection with FIG. 19A. Also shown in FIG. 19B are four cutaway portions, one of which labeled 1205, that are situated alongside the heaters that are situated along the long side of the PCR reaction zone. (Cutaways 1205 are shown cross-hatched in the upper panel of FIG. 19B, even though they are shown as clear (no crosshatching) in the lower panel.) Cutaway portions such as 1205 reduce the thermal mass of the heater substrate, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction zone. Four separate cutaway portions are shown in FIG. 19B. Multiple cutaway portions are typically used so that control circuitry to the various heaters is not disrupted. Other configurations of cutouts, such as in shape, position, and number, are consistent with the present technology. These cutouts may be created by 20 a method selected from: selective etching using wet etching processes, deep reactive ion etching, selective etching using CO<sub>2</sub> laser or femtosecond laser (to prevent surface cracks or stress near the surface), selective mechanical drilling, selective ultrasonic drilling, or selective abrasive particle blasting. 25 Care has to be taken to maintain mechanical integrity of the heater while reducing as much material as possible.

FIG. 19C shows a combination of cutouts in cartridge (comprising substrate 923 and laminate layer 925) and heater substrate 921, and use of ambient air cooling to increase the 30 cooling rate during the cooling stages of thermocycling. A substantial amount of cooling happens by convective loss from the bottom surface of the heater surface to ambient air. The driving force for this convective loss is the differential in temperatures between the heater substrate surface (typically 35 made of glass) and the air temperature. By decreasing the ambient air temperature by use of, for example, a peltier cooler 1207, the rate of cooling can be increased. The convective heat loss may also be increased by keeping the air at a velocity higher than zero. Peltier cooler 1207 is situated 40 beneath a printed circuit board, layer 927 as shown in crosssection in FIG. 19C. Peltier cooler comprises a cooling surface 1209, a number of p-n junctions 1211 on a substrate 1213. The substrate itself can be mounted on a finned heat sink 1215, in which are one or more cutouts 1217 to facilitate 45 cooling

An example of thermal cycling performance obtained with a configuration as described herein, is shown in FIG. **20** for a protocol that is set to heat up to 92° C., and stay there for 1 second, then cool to 62° C., and stay for 10 seconds. Cycle 50 time is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C.

Highly Multiplexed Embodiments

Embodiments of the apparatus and cartridge described 55 herein may be constructed that utilize high-density microfluidic circuitry on a single cartridge that thereby permit processing of multiple samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 36, 40, 48, 50, 64, 72, 80, 84, 96, and 100, but it would 60 be understood that still other numbers are consistent with the apparatus and cartridge herein, where deemed convenient and practical.

Accordingly, different configurations of lanes, sample inlets, and associated heater networks that can facilitate processing such numbers of samples on a single cartridge are within the scope of the instant disclosure.

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In an exemplary embodiment, shown in FIGS. 21A-21C, a highly multiplexed cartridge has 48 lanes. FIG. 21A shows a plan view of a representative 48-lane cartridge. FIG. 21B shows, in close up, an exemplary spacing of valves and lanes in adjacent lanes of a multi-sample microfluidic cartridge.

FIG. 21C shows an inlet configuration for accepting 48 samples. The inlet configuration is compatible with an automatic pipetting machine that has dispensing heads situated at a 8 mm spacing. For example, such a machine having 4 heads can load 4 inlets of the cartridge of FIGS. 21A and 21C at once, in 12 discrete steps.

Each lane of the cartridge has a microfluidic network that includes a PCR chamber and has independently controllable valves in one or more channels, with 2 banks of thermocycling protocols per channel, as shown in FIG. 22A. In the embodiment in FIG. 22A, the heaters are arranged in three arrays. Heaters in two separate glass regions only apply heat to valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and passed to the PCR reaction chambers independently of one another. The PCR heaters are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. In some embodiments, the PCR heaters are arranged in 2 banks (the heater arrays on the left and right are not in electrical communication with one another), thereby permitting a separate degree of sample control.

FIGS. 22A and 22B show heater arrays of the exemplary cartridge of FIG. 21A in, respectively, plan view, and in close-up.

FIGS. 23A-23C show various views of an embodiment of a radially-configured highly-multiplexed cartridge, having a number of inlets, microfluidic lanes, and PCR reaction zones.

The various embodiments shown in FIGS. 21A-23C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the specific examples described herein.

In another embodiment (not shown in the FIGs.), a cartridge and apparatus is configured so that the read-head does not cover the sample inlets, thereby permitting loading of separate samples while other samples are undergoing PCR thermocycling.

Heater Multiplexing (Under Software Control)

Another aspect of the apparatus described herein, relates to a method for controlling the heat within the system and its components. The method leads to a greater energy efficiency of the apparatus described herein, because not all heaters are heating at the same time, and a given heater is receiving current for only part of the time.

Generally, the heating of microfluidic components, such as a PCR reaction zone, is controlled by passing currents through suitably configured microfabricated heaters, as illustrated in FIG. 4E. The heating can be further controlled by periodically turning the current on and off with varying pulse width modulation (PWM), wherein pulse width modulation refers to the on-time/off-time ratio for the current. The current can be supplied by connecting a microfabricated heater to a high voltage source (for example, 30V), which can be gated by the PWM signal. In some embodiments, the device includes 48 PWM signal generators. Operation of a PWM generator includes generating a signal with a chosen, programmable period (the end count) and granularity. For instance, the signal can be 4000 μs (micro-seconds) with a granularity of 1 μs, in which case the PWM generator can

maintain a counter beginning at zero and advancing in increments of 1  $\mu s$  until it reaches 4000  $\mu s$ , when it returns to zero. Thus, the amount of heat produced can be adjusted by adjusting the end count. A high end count corresponds to a greater length of time during which the microfabricated heater receives current and therefore a greater amount of heat produced

In various embodiments, the operation of a PWM generator can also include a programmable start count in addition to the aforementioned end count and granularity. In such embodiments, multiple PWM generators can produce signals that can be selectively non-overlapping (e.g., by multiplexing the on-time of the various heaters) such that the current capacity of the high voltage power is not exceeded. Multiple heaters can be controlled by different PWM signal generators with varying start and end counts. The heaters can be divided into banks, whereby a bank defines a group of heaters of the same start count.

For example, 36 PWM generators can be grouped into six different banks, each corresponding to a certain portion of the PWM cycle (500 ms for this example). The end count for each PWM generator can be selectively programmed such that not more than six heaters will be on at any given time. A portion of a PWM cycle can be selected as dead time (count 3000 to 4000 for this example) during which no heating takes place and sensitive temperature sensing circuits can use this time to sense the temperature. The table below represents a PWM cycle for the foregoing example:

	Start Count	End Count	Max End Count
	Ba	nk 1	
PWM generator#1	0	150	500
PWM generator#2	0	220	500
PWM generator#6		376	500
	Ва	nk 2	
PWM generator#7 PWM generator#8	500 500	704 676	1000 1000
r w w generator#8			1000
PWM generator#12	500	780	1000
	Ba	nk 3	
DW/M conceptor#12	1000	1240	1500
PWM generator#13 PWM generator#14	1000	1101	1500
···			
PWM generator#18	1000	1409	1500
	Ba	nk 4	
PWM generator#19	1500	1679	2000
PWM generator#20	1500	1989	2000
PWM generator#24	1500 Par	1502 nk 5	2000
	Ба	IIK 3	
PWM generator#25	2000	2090	2500
PWM generator#26	2000	2499	2500
DILLO 6 4 420	2000	2201	2500
PWM generator#30	2000 Ba	2301 nk 6	2500
	Da		
PWM generator#31	2500	2569	3000
PWM generator#32	2500	2790	3000
PWM generator#36	2500	2678	3000

Use of Detection System to Measure/Detect Fluid in PCR Chamber

The apparatus optionally has a very sensitive fluorescence detector that is able to collect fluorescence light from the PCR

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chamber 210 of a microfluidic cartridge. Further aspects of such a detector are described in U.S. patent application Ser. No. 12/173,023 (now U.S. Pat. No. 8,133,671), filed Jul. 14, 2008, and incorporated herein by reference. Such a detector is used to monitor the progress of PCR, and can also be used to detect the presence of liquid in the chamber, a measurement that determines whether or not to carry out a PCR cycle on that particular chamber. The detector can be, for example, an optical detector having a light source (for example an LED) that selectively emits light in an absorption band of a fluorescent dye, lenses for focusing the light, and a light detector (for example a photodiode) that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof. One way to accomplish this determination is to take a background reading prior to filling the chamber with liquid. Another reading is taken after operations have been performed that should result in filling the PCR chamber with liquid. The presence of liquid alters the fluorescence reading from the chamber relative to an empty chamber. A programmable threshold value is used to tune an algorithm programmed into the processor (for example, the second reading has to exceed the first reading by 20%). If the two readings do not differ beyond the programmed margin, the liquid is deemed to not have entered the chamber, and a PCR cycle is not initiated for that chamber. Instead, a warning is issued to a user.

### Liquid Dispenser

The microfluidic cartridge is configured to receive sample(s) via the one or more inlets, and delivered by a liquid dispenser. A suitable liquid dispenser for use with the apparatus herein is described in U.S. patent application Ser. No. 12/212,403, filed Sep. 17, 2008, and incorporated herein by reference.

In various embodiments, preparation of a PCR-ready sample for use in subsequent diagnosis using the apparatus as further described herein, can include one or more of the following steps: contacting a neutralized polynucleotide 40 sample with a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid); in some embodiments, the 45 PCR reagent mixture can be in the form of one or more lyophilized pellets, as stored in a receptacle on a holder, and the method can further include reconstituting the PCR pellet with liquid to create a PCR reagent mixture solution. Various, such as one or more, of the liquid transfer operations associ-50 ated with the foregoing steps can be accomplished by an automated pipette head.

During sample preparation, the liquid dispenser is configured to carry out fluid transfer operations on two or more holders simultaneously, and can be mounted on a gantry 55 having three degrees of translational freedom.

The liquid dispenser typically comprises a number of individually sprung heads, wherein each head is configured to accept a pipette tip from the one or more pipette tips in a holder, and to control dispense operations with that pipette tip. For example, a typical liquid dispenser comprises four individually sprung heads, but it is to be understood that the dispenser is not limited to this number. For example, other numbers include 2, 3, 5, 6, 8, 10, or 12. The liquid dispenser can be further configured such that no two heads accept pipette tips from the same holder. The individually sprung heads may be arranged in parallel to one another, but may be configured in other arrangements.

The liquid dispenser is further configured to aspirate or dispense fluid in connection with analysis or preparation of solutions of two or more samples. The liquid dispenser is also configured to dispense liquid into a microfluidic cartridge. Additionally, the liquid dispenser is configured to accept or 5 dispense, in a single operation, an amount of 1.0 ml of fluid or less, such as an amount of fluid in the range 10 nl-1 ml.

Typically when transferring a sample containing extracted nucleic acid from a pipette tip to an inlet on the microfluidic cartridge, say using a liquid dispenser, a volume of air is 10 simultaneously introduced into the microfluidic network that is connected to the inlet, the volume of air being typically between about 0.5 mL and about 5 mL, but depending on the volume of the pipette tip. Presence of air in the microfluidic network, however, is not essential to operation of the cartridge described herein.

Proper alignment of pipette tips attached to the dispense head with the inlets on the cartridge can be achieved with a motorized alignment plate, as further described in U.S. patent application Ser. No. 12/212,403, filed Sep. 17, 2008, and 20 incorporated herein by reference.

Cartridge Autoloader

In certain embodiments of the apparatus described elsewhere herein, microfluidic cartridges are stored in a loading device and automatically delivered to a receiving bay when 25 needed. Such an arrangement, which may be referred to as an amplification-detection system, permits multiple samples to be analyzed, in batches, and with minimal interruption between successive cartridges.

An exemplary embodiment of an amplification-detection system 2900 for use with a microfluidic cartridge is shown in FIG. 24. The system 2900 performs and automates the process of PCR on multiple nucleic-acid containing samples, on a cartridge, in parallel. The system 2900 comprises a repository 2907 for unused microfluidic cartridges, a cartridge autoloader, a receiving bay 2902 for a microfluidic cartridge, a detector 2908, and a waste tray 2903 configured to receive used microfluidic cartridges. In one embodiment, the cartridge autoloader comprises a cartridge pack 2901, and a cartridge pusher 2904.

The system 2900, for illustration purposes, is configured so that a microfluidic cartridge moves in a plane and linearly from the repository 2907 to the receiving bay 2902, to the waste bin 2903, but it need not be so arranged. For example, the waste cartridge bin 2903 can be aligned orthogonally, or any angle thereof, to the receiving bay, such as disposed behind it. Alternatively, each element (cartridge autoloader 2901, receiving bay 2902, and waste cartridge bin 2903) can be configured in a step-wise manner where the cartridge pack 2901 is on the same, higher or lower level than the amplification-detection system 2902 and the amplification-detection system 2902 is on the same, higher or lower level than the waste cartridge bin 2903. Another configuration could be that each of the three elements is not arranged linearly but at an angle to one another, although within the same plane.

FIG. 24 illustrates the cartridge pack 2901 and the waste cartridge bin 2903 below the plane of the receiving bay, and a detection system 2908 above the plane. This configuration is exemplary and it would be understood that some of these elements may be positioned alternately above or below the 60 plane in other embodiments.

FIG. 25 illustrates a repository for unused microfluidic cartridges. The repository can be configured to accept a number of individually stacked and individually loaded cartridges, or can be configured to accept a pack of cartridges 65 2901, where it is to be understood that a pack is a set of two or more cartridges that are stored and transported together with

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one another, even though each will be used separately. An exemplary cartridge pack has 24 cartridges. The repository may consist of a cage **2910** of any material that may or may not be transparent. For example it may be made of metal or plastic. The cartridge pack **2901** is not limited to twenty-four cartridges **2906** per pack but may contain any number from 2 to 100. For example, other numbers such as 2, 4, 8, 10, 12, 16, 20, 30, 36, 40, 48, 50, or 64 are possible numbers of cartridges per pack. Similarly, the repository may be configured to accept those numbers of cartridges, when individually stacked, rather than being in a pack.

In some embodiments, as in FIG. 25, each cartridge 2906, individually stacked, rests so that ridges along two sides of the cartridge are supported on ledges 2911 that protrude from the cage 2910. However, other configurations are possible. For example, a cartridge 2906 may rest on recessed grooves made within the interior surfaces of cage 2910. Furthermore, the cartridge pack 2901 may not need to be placed in a cage 2910. The cartridge pack 2901 may itself include the necessary connections to bind securely to the apparatus and to load the cartridges 2906.

FIG. 26 is an illustration of an exemplary initial loading position of a cartridge pack 2901 in a depository when samples are loaded in the topmost cartridge in the pack. FIG. 26 shows the cartridge pack 2901 below a plane that contains a cartridge pusher. In other embodiments, the cartridge pack 2901 may be above the plane of a cartridge pusher where the pusher pushes the lowest cartridge out from the holder; or partly above and partly below in a holder 2920 where a cartridge pusher pushes a cartridge from the middle of the cartridge pack 2901. In the embodiment shown in FIG. 26, a topmost cartridge 106 is pushed along two guide rails 2905. Alternatively, there may be more or fewer guide rails (such as one or three) or no guide rails at all so long as a cartridge 2906 can be caused to move to other required positions with reliability and accuracy of positioning.

An exemplary cartridge pusher 2904 is shown in FIG. 27. The cartridge pusher 2904 pushes a cartridge 2906 along guide rails 2905, which allows a cartridge 2906 to travel to pre-calibrated positions by the mechanism of a stepper motor 2930. However, it would be understood that the mechanism of transporting the cartridge 2906 is not limited to a stepper motor 2930 and thus other mechanisms are also consistent with the cartridge pusher 2904 as described herein.

FIG. 28 shows a used cartridge 2906 that has been pushed by the cartridge pusher 2904 into the waste cartridge bin 2903 after a PCR process has been completed, such as on multiple samples in the cartridge. The embodiment shows a lipped handle 2940 that facilitates easy handling, such as emptying, of the bin 2903. However, it would be understood that the handle 2904 is not limited to the style and shape shown.

An exemplary cartridge pack 2901, before and after multiple PCR processes are completed, is shown in FIG. 29. After the cartridge pusher 2904 pushes a cartridge 2906 out of the cartridge pack 2901, a spring 2950 at the bottom of the cartridge pack pushes against the lower surface of the stack of cartridges and causes the topmost cartridge to be made available for sample injection. The spring 2950 is not limited in number or type. Thus although a single helical or coiled spring is shown, it is consistent with the description herein that more than one helical or coiled springs could be used, such as 2, 3, or 4, and that alternatively a sprung metal strip, or several strips, could be used. Alternatively another mechanism for forcing the cartridges upwards could be deployed, such as a pneumatic, hydraulic, or inflatable pressurized container, could be utilized.

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The cartridge pushing mechanism can also be made to not only push the cartridge from the autoloader box to the detection position, but also be used to move it back to the autoloading position. This will enable unused lanes in the microfluidic cartridge to be used in the next PCR run.

The cartridge autoloading box is also designed so that once all the cartridges are used, the box can be easily recycled or new cartridges added to it. This reduces the cost to the customer and the manufacturer.

It is to be noted that microfluidic cartridges, as further 10 described herein, that have a raised lip along their edges to permit ease of stacking and/or storage in a pack or an autoloader are particularly advantageous because the raised lips also introduce a stiffness into the cartridges and assist in keeping the fluid inlets on one cartridge away from those on 15 another cartridge during storage and transport. The raised regions, which need not only be lips along each edge of a cartridge, also help minimize friction between the lower surface of one cartridge and the upper surface of another during transport and use.

Sample Preparation, and Reagents

In various embodiments, the PCR-ready sample injected into the cartridge can include a PCR reagent mixture comprising a polymerase enzyme, and a nucleotide or a plurality of nucleotides from a biological sample in question. The PCR 25 reagent mixture can be in the form of one or more lyophilized pellets prior to mixing with the sample, and the steps by which the PCR-ready sample is prepared can involve contacting the PCR pellet with liquid to create a PCR reagent mixture solution. In yet another embodiment, each of the PCR chambers in the cartridge may have dried down or lyophilized ASR reagents preloaded such that the user only needs to input prepared polynucleotide sample into the cartridge. In another embodiment, the PCR chambers of the cartridge may have only the application-specific probes and primers premeasured 35 and preloaded, and the user inputs a sample mixed with the PCR reagents.

In various embodiments, the PCR-ready sample further includes a sample buffer, and at least one probe that is selective for a polynucleotide sequence, e.g., the polynucleotide 40 sequence that is characteristic of a pathogen selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the sample can include at least one probe that can be selective for a polynucleotide sequence. 45 The probe can be a fluorogenic hybridization probe. The fluorogenic hybridization probe can include a polynucleotide sequence coupled to a fluorescent reporter dye and a fluorescence quencher dye.

In various embodiments, the PCR ready sample can further 50 include a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid, and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe. 55

In various embodiments, the microfluidic cartridge can accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent 60 mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide.

Each lane of a multi-lane cartridge as described herein can 65 perform two reactions because of the presence of two fluorescence detection systems per lane. A variety of combina-

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tions of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two lanes, such as adjacent lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

Carrying out PCR on a PCR-ready sample can include heating the PCR reagent mixture and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample; contacting the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; independently contacting each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; and/or contacting the neutralized polynucleotide sample or a PCR amplicon thereof and the negative 20 control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence.

In various embodiments, a method of using the apparatus described herein can further include one or more of the following steps: determining the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; determining a contaminated result if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof; and/or in some embodiments, wherein the PCR reagent mixture further comprises a positive control plasmid and a plasmid probe selective for at least a portion of the plasmid, the method further including determining that a PCR amplification has occurred if the plasmid probe is detected.

In various embodiments, the probes used can be selective for a polynucleotide sequence that is characteristic of an organism, for example any organism that employs deoxyribonucleic acid or ribonucleic acid polynucleotides. Thus, the probe can be selective for any organism. Suitable organisms include mammals (including humans), birds, reptiles, amphibians, fish, domesticated animals, wild animals, extinct organisms, bacteria, fungi, viruses, plants, and the like. The probe can also be selective for components of organisms that employ their own polynucleotides, for example mitochondria. In some embodiments, the probe is selective for microorganisms, for example, organisms used in food production (for example, yeasts employed in fermented products, molds or bacteria employed in cheeses, and the like) or pathogens (e.g., of humans, domesticated or wild mammals, domesticated or wild birds, and the like). In some embodiments, the probe is selective for organisms selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of *Staphylococcus* spp., e.g., *S. epidermidis*, *S. aureus*, Methicillin-resistant *Staphylococcus* aureus (MRSA), Vancomycin-resistant *Staphylococcus*; *Streptococcus* (e.g., α, β or γ-hemolytic, Group A, B, C, D or G) such as *S. pyogenes*, *S. agalactiae*; *E. faecalis*, *E. durans*, and *E. faecium* (formerly *S. faecalis*, *S. durans*, *S. faecium*); nonenterococcal group D streptococci, e.g., *S. bovis* and *S. equines*; *Streptococci viridans*, e.g., *S. mutans*, *S. sanguis*, *S. salivarius*, *S. mitior*, *A. milleri*, *S. constellatus*, *S. intermedius*, and *S. anginosus*; *S. iniae*; *S. pneumoniae*; *Neisseria*, e.g., *N. meningitides*, *N. gonorrhoeae*, saprophytic

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Neisseria sp; Erysipelothrix, e.g., E. rhusiopathiae; Listeria spp., e.g., L. monocytogenes, rarely L. ivanovii and L. seeligeri; Bacillus, e.g., B. anthracis, B. cereus, B. subtilis, B. subtilus niger, B. thuringiensis; Nocardia asteroids; Legionella, e.g., L. pneumonophilia, Pneumocystis, e.g., P. 5 carinii; Enterobacteriaceae such as Salmonella, Shigella, Escherichia (e.g., E. coli, E. coli O157:H7); Klebsiella, Enterobacter, Serratia, Proteus, Morganella, Providencia, Yersinia, and the like, e.g., Salmonella, e.g., S. typhi S. paratyphi A, B (S. schottmuellerii, and C (S. hirschfeldii, S. 10 dublin S. choleraesuis, S. enteritidis, S. typhimurium, S. heidelberg, S. newport, S. infantis, S. agona, S. montevideo, and S. saint-Paul; Shigella e.g., subgroups: A, B, C, and D, such as S. flexneri, S. sonnei, S. boydii, S. dysenteriae; Proteus (P. mirabilis, P. vulgaris, and P. myxofaciens), Mor- 15 ganella (M. morganii); Providencia (P. rettgeri, P. alcalifaciens, and P. stuartii); Yersinia, e.g., Y. pestis, Y. enterocolitica; Haemophilus, e.g., H. influenzae, H. parainfluenzae H. aphrophilus, H. ducreyi; Brucella, e.g., B. abortus, B. melitensis, B. suis, B. canis; Francisella, e.g., F. tula- 20 rensis; Pseudomonas, e.g., P. aeruginosa, P. paucimobilis, P. putida, P. fluorescens, P. acidovorans, Burkholderia (Pseudomonas) pseudomallei, Burkholderia Burkholderia cepacia and Stenotrophomonas maltophilia; Campylobacter, e.g., C. fetus fetus, C. jejuni, C. pylori (Heli- 25 cobacter pylori); Vibrio, e.g., V. cholerae, V. parahaemolyticus, V. mimicus, V. alginolyticus, V. hollisae, V. vulnificus, and the nonagglutinable vibrios; Clostridia, e.g., C. perfringens, C. tetani, C. difficile, C. botulinum; Actinomyces, e.g., A. israelii; Bacteroides, e.g., B. fragilis, B. thetaiotaomicron, B. 30 distasonis, B. vulgatus, B. ovatus, B. caccae, and B. merdae; Prevotella, e.g., P. melaminogenica: genus Fusobacterium; Treponema, e.g. T. pallidum subspecies endemicum, T. pallidum subspecies pertenue, T. carateum, and T. pallidum subspecies pallidum; genus Borrelia, e.g., B. burgdorferi; genus 35 Leptospira; Streptobacillus, e.g., S. moniliformis; Spirillum, e.g., S. minus; Mycobacterium, e.g., M. tuberculosis, M. bovis, M. africanum, M. avium M. intracellulare, M. kansasii, M. xenopi, M. marinum, M. ulcerans, the M. fortuitum complex (M. fortuitum and M. cheloneii, M. leprae, M. asiaticum, 40 M. chelonei subsp. abscessus, M. fallax, M. fortuitum, M. malmoense, M. shimoidei, M. simiae, M. szulgai, M. xenopi; Mycoplasma, e.g., M. hominis, M. orale, M. salivarium, M. fermentans, M. pneumoniae, M. bovis, M. tuberculosis, M. avium, M. leprae; Mycoplasma, e.g., M. genitalium; Urea- 45 plasma, e.g., U. urealyticum; Trichomonas, e.g., T. vaginalis; Cryptococcus, e.g., C. neoformans; Histoplasma, e.g., H. capsulatum; Candida, e.g., C. albicans; Aspergillus sp; Coccidioides, e.g., C. immitis; Blastomyces, e.g. B. dermatitidis; Paracoccidioides, e.g., P. brasiliensis; Penicillium, e.g., P. 50 mameffei; Sporothrix, e.g., S. schenckii; Rhizopus, Rhizomucor, Absidia, and Basidiobolus; diseases caused by Bipolaris, Cladophialophora, Cladosporium, Drechslera, Exophiala, Fonsecaea, Phialophora, Xylohypha, Ochroconis, Rhinocladiella, Scolecobasidium, and Wangiella; Trichosporon, e.g., 55 T. beigelii; Blastoschizomyces, e.g., B. capitatus; Plasmodium, e.g., P. falciparum, P. vivax, P. ovale, and P. malariae; Babesia sp; protozoa of the genus Trypanosoma, e.g., T. cruzi; Leishmania, e.g., L. donovani, L. major L. tropica, L. mexicana, L. braziliensis, L. viannia braziliensis; Toxo- 60 plasma, e.g., T. gondii; Amoebas of the genera Naegleria or Acanthamoeba; Entamoeba histolytica; Giardia lamblia; genus Cryptosporidium, e.g., C. parvum; Isospora belli; Cyclospora cayetanensis; Ascaris lumbricoides; Trichuris trichiura; Ancylostoma duodenale or Necator americanus; 65 Strongyloides stercoralis Toxocara, e.g., T. canis, T. cati; Baylisascaris, e.g., B. procyonis; Trichinella, e.g., T. spiralis;

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Dracunculus, e.g., D. medinensis: genus Filarioidea; Wuchereria bancrofti; Brugia, e.g., B. malayi, or B. timori; Onchocerca volvulus; Loa boa; Dirofilaria immitis; genus Schistosoma, e.g., S. japonicum, S. mansoni, S. mekongi, S. intercalatum, S. haematobium; Paragonimus, e.g., P. Westermani, P. Skriabini; Clonorchis sinensis; Fasciola hepatica; Opisthorchis sp: Fasciolopsis buski: Diphyllobothrium latum; Taenia, e.g., T. saginata, T. solium; Echinococcus, e.g., E. granulosus, E. multilocularis; Picornaviruses, rhinoviruses echoviruses, coxsackieviruses, influenza virus; paramyxoviruses, e.g., types 1, 2, 3, and 4; adnoviruses; Herpesviruses, e.g., HSV-1 and HSV-2; varicella-zoster virus; human T-lymphotrophic virus (type I and type II); Arboviruses and Arenaviruses; Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae; Flavivirus; Hantavirus; Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis]); Viral hemorrhagic fevers (filoviruses such as Ebola, Marburg and arenaviruses such as Lassa, Machupo); Smallpox (variola); retroviruses e.g., human immunodeficiency viruses 1 and 2; human papillomavirus (HPV) types 6, 11, 16, 18, 31, 33, and 35.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytoca, Klebsiella pneumoniae, Escherichia coli, Acinetobacter Baumannii, Serratia marcescens, Enterobacter aerogenes, Enterococcus faecium, vancomycin-resistant enterococcus (VRE), Staphylococcus methecillin-resistant Staphylococcus (MRSA), Streptococcus viridans, Listeria monocytogenes, Enterococcus spp., Streptococcus Group B, Streptococcus Group C, Streptococcus Group G, Streptococcus Group F, Enterococcus faecalis, Streptococcus pneumoniae, Staphylococcus epidermidis, Gardenerella vaginalis, Micrococcus sps., Haemophilus influenzae, Neisseria gonorrhoeee, Moraxella catarrahlis, Salmonella sps., Chlamydia trachomatis, Peptostreptococcus productus, Peptostreptococcus anaerobius, Lactobacillus ferrmentum, Eubacterium lentum, Candida glabrata, Candida albicans, Chlamydia spp., Camplobacter spp., Salmonella spp., smallpox (variola major), Yersina Pestis, Herpes Simplex Virus I (HSV I), and Herpes Simplex Virus II (HSV II).

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of Group B *Streptococcus*.

### Computer Program Product

In various embodiments, a computer program product, such as a computer readable medium, for use with the apparatus herein includes computer readable instructions thereon for execution by a processor in connection with operating the apparatus.

In various embodiments, the computer program product can include one or more instructions to cause the system to: output an indicator of the placement of the microfluidic cartridge in the bay; read a sample label or a microfluidic cartridge label; output directions for a user to input a sample identifier; output directions for a user to load a sample transfer member with the PCR-ready sample; output directions for a user to introduce the PCR-ready sample into the microfluidic cartridge; output directions for a user to place the microfluidic cartridge in the receiving bay; output directions for a user to close the lid to operate the force member; output directions for a user to pressurize the PCR-ready sample in the microfluidic cartridge by injecting the PCR-ready sample with a

35 ne of air between about 0.:

volume of air between about 0.5 mL and about 5 mL; and output status information for sample progress from one or more lanes of the cartridge.

In various embodiments, the computer program product can include one or more instructions to cause the system to: 5 heat the PCR ready-sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; independently 10 contact each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control 15 polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; output a determination of the presence of a polynucleotide sequence in 20 the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; and/ or output a determination of a contaminated result if the probe is detected in the negative control polynucleotide or a PCR 25 amplicon thereof.

In various embodiments, the computer readable instructions are configured to independently actuate one or more microfluidic components (such as valves) of each lane in the microfluidic cartridge, independently of one another, and for 30 causing a detector to measure fluorescence from the PCR reaction zones.

In various embodiments, the computer readable instructions present a user with the option to either get results from all samples as quickly as possible, or from a first batch of 35 samples as quickly as possible and the subsequent batch later on.

### **EXAMPLES**

### Example 1

### Exemplary 3-Layer Cartridge

This example includes exemplary specifications used to 45 design and assemble a microfluidic cartridge as well as exemplary instructions on the use of the cartridge in, for example, the apparatus described elsewhere herein.

Characteristics

In some embodiments, the cartridge has the following 50 functional specifications.

The cartridge includes fluidic components (e.g., microchannels, valves, end vents, reagent inlet holes, reaction chambers, and the like) necessary to perform the functions of the cartridge (e.g., PCR).

The cartridge can be adapted to a one-time use, making it a disposable cartridge that can be disposed of according to typical laboratory procedures.

The cartridge is 4.375 inches long and 2.800 inches wide, with a thickness of 0.094+/-0.005 inches. The cartridge 60 includes features that allow it to interface with, for example, the system described herein. The interfacing features include PCR channel walls and the top of the micro-substrate over the PCR channel that are well polished (SP1A1/A2/A3), enabling easy transfer of excitation and emission light between the 65 PCR reactor (contained in the cartridge) and a detection system. The cartridge includes a thermal interface, located on the

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bottom of the cartridge, for interfacing with the analyzer. The thermal interface includes a thin laminate (typically less than 150 microns thick, such as 100 microns thick) to encourage heat transfer from the heater wafer to, for example, the PCR channels of the cartridge.

The cartridge includes one or more mechanical interfaces with, for example, the receiving bay of a diagnostic apparatus as described elsewhere herein. For example, the cartridge has a notch in one or more of the corners that can mate with a corresponding shape on the heater module of the receiving bay. The notch and corresponding shape enables the cartridge to be placed only one way in the receiving bay. In some embodiments, the cartridge has a single notch in one of the corners, with the remaining three corners having a minimum radius of 1 mm to facilitate placement of the cartridge in the analyzer.

The cartridge includes a number of inlet holes that are cone-shaped with a height of 1 mm from the top surface of the cartridge. The cone has an inner diameter of 3 mm at the top of the cone and tapers uniformly down to a diameter that matches the width of a microchannel (e.g., an inlet channel) to which the inlet cone is fluidly connected. The inlet channel fluidly connects the inlet hole to a PCR reactor that has an interior volume of, for example, about 4.25  $\mu$ l to 4.75  $\mu$ l (e.g., 4.22  $\mu$ l, 4.5  $\mu$ l, 4.75  $\mu$ l, or the like). An outlet microfluidic channel fluidly connects the PCR reactor to an overflow chamber. The cartridge also includes an outlet vent hole.

The microfluidic substrate layer of the cartridge can include one or more of the following specifications. The material of the microsubstrate is optically clear (e.g., has about 90% or greater optical transmission, be 3 mm thick, comply with ASTMD1003, and the like), have auto-fluorescence that is less than that emitted by 2 mm thick ZEONOR 1420R, and have a refractive index of about 1.53 (ASTMD542). The material of the microsubstrate is amenable to the injection molding of features required for the microfluidic networks of the cartridge. The material is typically compatible with all PCR agents and can withstand temperatures of up to about 130° C. for about 5 minutes or more without yielding or melting.

The cartridge includes fiducials, recognizable by suitably configured manufacturing equipment, located in one or more (preferably two) of the corners of the substrate.

Additional features of the substrate material include one or more of the following. Minimum clearances of about 1 mm are present between adjacent functional features to ensure sealing success (e.g., to the analyzer), and to allow simplified fixturing during assembly. The cartridge can include "dogbones" under small fluid path ends to, for example, increase mold life. The bottom surface of the cartridge can be roughened (e.g., by vapor hone, EDM, or the like prior to attaching laminate to the substrate). The substrate material is capable of adhesion by a label.

In some embodiments, the sealing tape used in the cartridge includes one or more of the following specifications. Laminate can be easily applied to the bottom of the microfluidic substrate. The material of the laminate is essentially pin-hole free. The material of the sealing tape, in particular the adhesive, are compatible with the PCR reaction chemistries. The laminate material and glue used should not autofluoresce. The material can withstand up to 130° C. for 5 minutes without losing adhesion, and without yielding, melting, or causing undue stresses on the cartridge. Bubbles should not form in the adhesive layer upon heating (e.g., to 130° C. for 5 minutes) after application to the microsubstrate.

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The laminate is less than 5 mils thick (typically 4 mils thick) to, for example, enable rapid heat transfer. (1 mil=1/1000 inch=25.4 microns.)

The high temperature wax included in the cartridge has the following characteristics. The wax should have a melt point of about  $90+/-3^{\circ}$  C. (e.g.,  $87^{\circ}$  C.,  $90^{\circ}$  C.,  $93.1^{\circ}$  C., or the like), be biocompatible with PCR reactions, have wettability with microsubstrate material, and have a melt viscosity range, for example, of about viscosity at  $100^{\circ}$  C.=20 mm²/s and Hardness at  $25^{\circ}$  C.=8 dmm.

The main label on the upper surface of the cartridge has the following characteristics. It has a thickness of 2-144 mils, has suitable bondability to micro features, and seals around the valves, includes cuts for one or more PCR windows, and optionally space for a tab (free from adhesive) for aiding in removal of the cartridge from the analyzer. In embodiments, where a lid of a receiving bay for the cartridge slides over the cartridge, the main label also has resistance to abrasion on its upper surface, and is printable. The main label can have an upper and lower alignment pattern for the label to completely cover the valve holes to permit proper operation of the valves.

The cartridge includes a barcode label applied to the top of the cartridge, which is readable by a barcode reader (e.g., the barcode reader included in the apparatus described elsewhere 25 herein) while the cartridge is installed in the analyzer. The barcode label can include the product name, lot #, expiration date, bar code (2D) and may be printed directly onto the cartridge. In addition, or in the alternative, a barcode may be applied directly to the main cartridge label using a laser or 30 inkjet type printer. The label is suitable for being read by a scanning head, for example as depicted in FIG. 30.

The packaging in which the cartridge is shipped includes one or more of the following: package label, carton, carton label, and/or operating instructions. The packaging can be 35 printed on or can have a label attached to it. The cartridge can be placed inside of a plastic bag, shrink/stretch wrap bag, or the like. The cartridge can be stacked and packaged in groups, such as of 12, or 24. It is not critical that the cartridge bagging has a seal but it should be kept free from contamination, such 40 as dust.

### Microfluidic Components

The cartridge includes microchannels and holes such that the holes are of a size and shape to enable easy, leak-free interfacing with a 175  $\mu$ l pipette tip. In some examples, the 45 hole size is between about 200  $\mu$ m and about 4000  $\mu$ m in diameter. The microchannels can be between about 50  $\mu$ m and about 1500  $\mu$ m wide and between about 50  $\mu$ m and 1000  $\mu$ m high.

The cartridge includes valves for controlling the flow of 50 fluid within the cartridge (e.g., through the microchannels, reactor chambers, and the like). The valve edges, steps, and general geometry can be designed to encourage exact flow and/or stoppage required during wax load. The valve geometry can be designed to accommodate limitations of wax 55 dispensing equipment (e.g., +/-25% of 75 mL volume). In some embodiments, step down air chambers on the valves are funnel shaped to aid wax loading and the remaining geometry diminishes from the bottom of the funnel to the end point where the wax stops. The path where the valves are to flow 60 into and block, during use, is narrow enough (e.g., 150-200 microns wide and deep), and has enough length, to effectively seal when the valves are activated during use. The valve wax temperature can be about 90° C. When in use to block a portion of a microchannel, the valves seal to prevent evapo- 65 ration of fluid and/or physical migration of fluid from the PCR reactor during thermocycling.

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The cartridge includes one or more valves (such as temperature controlled, wax-containing valves) for starting, stopping, and/or controlling the flow of material inside the cartridge. The wax contained in the valves is free of trapped air bubbles that have a diameter greater than half the width of the valve channel. The valve channel can have an air pocket. The wax may not intrude into the fluid path (the  $150\mu$  channel) prior to activation. The wax can be filled to the start of the flare at the fluid path (i.e., the junction of the  $150\mu$  fluid channel and the wax loading channel, see, e.g., FIG. 9A).

The cartridge can include one or more PCR regions for performing PCR on a sample. The channel in the PCR region (the PCR reactor) can be designed such that the temperature of the contents of the channel remain uniformly within about 1° C. of the PCR anneal temperature. The channel walls can have a polish of SP1A1/A2/A3.

Operation

An input PCR sample (e.g., a reaction mixture) can be between about 6.0 and 7.0 µl per PCR lane (e.g., 5.9 µl per lane, 6.4 µl per lane, 7.1 µl per lane, or the like) and is introduced into the cartridge through the inlet hole by, for example, a pipette. A reaction mixture is transported, via the inlet channel, to the PCR reactor where the reaction mixture is isolated (e.g., sealed off by valves) to prevent evaporation or movement (leakage) of the reaction mixture during thermocycling. Once the various reaction mixtures are sealed inside their respective chambers, the diagnostic apparatus initiates multiplexed real-time PCR on some or all of the reaction mixtures (e.g., 4.5 µl, an amount of fluid equal to the inner volume of the reaction chamber, or the like).

In some embodiments, the cartridge is designed to be able to perform diagnostic tests within a temperature range of about 59° F. to about 86° F. (about 15° C. to about 30° C.) and a humidity range of about 15% relative humidity to about 80% relative humidity. The cartridge is designed to be safe and functional when used indoors, used at an altitude of 2000 m or less, and used under non-condensing humidity conditions (e.g., maximum relative humidity of 80% for temperatures up to 31° C. decreasing linearly to 50% relative humidity at 40° C.).

In use, end-product of PCR, produced in the cartridge, can remain in the used cartridge to, for example, minimize the likelihood of cross contamination.

The cartridge can be designed such that a drop from a height of 4 foot, while in its packaging, will not damage the cartridge. The cartridge is designed to perform without damage after exposure to the following conditions. The cartridge should be stored at 4° C. to 40° C. for the rated shelf life. Exposure to temperatures between –20° C. and 4° C. or 40° C. and 60° C. should occur for no longer than 24 hours. The cartridge can withstand air pressure changes typical of air transport.

The cartridge can be labeled with the following information (e.g., to identify the cartridge, comply with regulations, and the like). The label can contain a "Research Use Only" label, if applicable, and a CE mark, if applicable. The label can contain the company name and logo (e.g., HandyLab®), a part number (e.g., 55000009), a part name (12x Cartridgenonvented), a lot number (e.g., LOT 123456), an expiration date (e.g., in month and year format, such as "06/2015"), space for writing, a barcode according to barcode specifications (described elsewhere), and/or manufacturer name and address, e.g., "Handylab, Inc., Ann Arbor, Mich. 48108 USA".

The cartridge can be provided in a carton that contains information on its exterior or interior such as: a part number (e.g., 55000009), a part name (12x Cartridge-nonvented), a

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quantity (e.g., 24), a lot number (e.g., LOT 123456), an expiration date (e.g., 06/2015), an optional UPC code, a manufacturer's statement and contact details, such as "Manufactured by Handylab, Inc., Ann Arbor, Mich. 48108 USA", a carton label to state storage limits, a CE mark (if applicable), 5 and/or an AR name and address.

The cartridge packaging can include paper wrap to secure multiple cartridges together, and clean package fill to prevent damage, for example, from vibration. The cartridge shipping carton can include features such as, compliance to ASTM 6159, carton may be stored in any direction, refrigeration or fragile labeling of the carton may not be required, and additional cold packs may not be required. The shelf life of the cartridge is 12 months or more.

The cartridge complies with 1EC 61010 (NRTL tested) and an FDA listing may be required for clinical distribution. Cartridges used in a clinical lab device typically meet all quality system requirements. Cartridges used for research only in a commercial device may meet all quality system requirements of a particular research institution, or HandyLab, Inc. Cartridges for research use only (Alpha or Beta testing) may be design/manufacturing traceable to a device history record (DHR) (such as a manufacturing record).

The cartridge has a maximum limit of detection equal to 20 copies per reaction volume (i.e., 20 copies/4 $\mu$ ) with a target detection of 10 copies per reaction volume. The cartridge can perform 45 reaction cycles in 40 minutes or less (e.g., 45 cycles in 40 minutes, 45 cycles in 20 minutes, 45 cycles in 15 minutes). The cartridge can utilize two color detection using, for example, the FAM (or equivalent) and CAL RED (or equivalent) fluorescent dyes. Results obtained using the cartridge have been compared with the results obtained using standard real-time PCR instruments.

During use (e.g., when placed in a system described herein and performing a function such as PCR), the cartridge is pressed, on one side, by the optics block, against the heater wafer (positioned against the opposite side), with a pressure of about 1 psi or greater (e.g., 0.99 psi, 1.2 psi, or the like). When located in the tray of the analyzer, the cartridge has an alignment slop of +/-200 microns to enable a user to easily place and remove the cartridge from the analyzer tray. The cartridge has two ledges, that are each 1 mm wide and located along the two long edges of the cartridge, to enable the heating (lower) surface of the cartridge to extend below the datum of the tray and therefore make effective contact with the upper surface of the heater unit.

Cartridge Manufacturing:

Existing semi-automatic equipment for laminating & waxing (Think & Tinker DF-4200, & Asymtek Axiom Heated Jet Platform, respectively) can be utilized to meet all cartridge manufacture requirements. An exemplary protocol is as follows:

Laminate micro substrate & trim excess.

Fill valves with hot wax & inspect.

Apply label & barcode.

Band 24 pieces together.

Bag & seal banded cartridges, label bag.

Place bag & insert(s) into carton, seal and label.

Venting is not required on this cartridge, which eliminates the most time consuming process for cartridge manufacture, along with the highest risk and highest cost for fully integrated automation. (In embodiments, where a hydrophobic 65 membrane needs to be attached, the membrane requires careful placement, alignment, and pressure sealing at a properly 40

regulated temperature.) Over 1,000 pieces of the 12-up without venting have been successfully produced.

#### Example 2

Exemplary Chemistry and Processes of Use

The chemistry typically performed in conjunction with the microfluidic cartridge herein centers around the detection and identification of organisms in a clinical specimen, by virtue of detecting nucleic acids from the organism in question. This involves isolation of nucleic acids from target organisms that are contained in a clinical specimen, followed by a process that will detect the presence of specific nucleic acid sequences. In addition to target detection, an internal positive control nucleic acid is added to the collection buffer, and is taken through the entire extraction and detection process along with target nucleic acids. This control monitors the effectiveness of the entire process and minimizes the risk of having false negative results.

Nucleic Acid Extraction and Purification

Nucleic acid extraction procedures begin with the addition of a clinical specimen to a prepared specimen collection solution. This can be done either at a specimen collection site, or at the testing site. Two collection solution formats are available: one for body fluids, and one for swab specimens. Collection solutions used at collection sites serve as specimen transport solutions, and therefore, this solution must maintain specimen and analyte integrity.

The extraction and purification procedure, which can be entirely automated, proceeds as follows:

Target organisms are lysed by heating the detergent-containing collection solution.

Magnetic beads, added to the specimen/collection solution 35 mix, non-specifically bind all DNA that is released into the solution.

Magnetic beads are isolated and are washed to eliminate contaminants DNA is released from the beads using high pH and heat.

DNA containing solution is removed and neutralized with a buffer

Nucleic Acid Amplification

Nucleic acids that have been captured by magnetic beads, washed, released in high pH, and neutralized with buffer, are added to a mixture of buffers, salts, and enzymes that have been lyophilized in a tube. The mixture is rapidly rehydrated, and then a portion of the solution is loaded onto a microfluidic cartridge. The cartridge is then loaded into the amplification instrument module, which consists of a heating unit capable of thermal cycling, and an optical detection system. Detection of target nucleic acids proceeds as follows:

The liquid is sealed in a reaction chamber.

Rapid thermal cycling is used to potentiate the Polymerase Chain Reaction (PCR), which is used to amplify specific 55 target DNA.

Amplified DNA fluoresces, and can be detected by optical sensors.

A fluorescent probe "tail" is incorporated into each amplified piece of DNA.

At a specific temperature, the probe adopts a conformation that produces fluorescence (this is termed a "scorpion" reaction).

Fluorescence is detected and monitored throughout the reaction.

55 Extraction and Amplification/Detection Process

Extensive bench-scale testing has been carried out to optimize the nucleic acid extraction chemistry, including the col-

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lection buffer, the wash buffer formulation, the release solution formulation, and the PCR reagent mixes. The fully automated method of extraction, followed by 12-up PCR, was able to provide very high sensitivity consistently at 150 copies/sample.

Examples: *Chlamydia* in Urine (50/50); Gonorrhoea in Urine; GBS in Plasma.

Various detection chemistries, such as Taqman, Scorpion, SYBRg Green, work reliably in the microfluidic cartridge. Reagent Manufacturing

Feasibility studies were conducted in order to determine whether PCR reagents could be lyophilized in PCR tubes besides the use of 2 µl lyophilized pellets. The studies have indicated that sensitivity of reactions performed using tubelyophilized reagents is equivalent to that of wet reagents or 2 µl pellet reagents, so feasibility has been proven. Stability studies for this format indicate similar stability data. 2 microliter lyophilized PCR pellets have been found to be stable to up to 2 years at room temperature, once sealed in nitrogen atmosphere.

There are currently seven individual, blended chemistry components identified for potential use with the system described herein. Mixing, blending and processing reagents/ chemicals can be performed at HandyLab, Inc., with existing equipment already in place.

Collection buffer, wash, release and neutralization liquids are simple recipes with very low risk, and can be made in large batches to keep labor costs of mixing/blending at or below targeted projections. They can be mixed and placed into intermediate containers for stock, and then issued to a manufacturer of reagent holders for dispensing.

Affinity Beads (AB) have good potential to be stored and used as a liquid in the strip, but design contingencies for using a lyophilized pellet are in place as a back up. It is important to keep the beads suspended in solution during dispense. Dispense equipment (e.g., manufactured by Innovadyne) that provides agitation for continuous suspension during dispense has been identified for achieving this, once stability has been proven for liquid AB storage in the strip. The process to manufacture and magnetize the Affinity Beads spans a 9 hour cycle time to produce a batch of 2,000 aliquots, but that same time period can be used for scaled up recipe batches once we ramp into high volume production. This item has the highest labor content of all chemistry manufacture that is currently required for the apparatus.

PCR reagents/enzymes can be freeze-dried in a lyophilizing chamber (e.g., Virtis Genesis) but will not require spherical pellet formation. Instead, the mixture can be dispensed into, and then lyophilized, inside the end-use tube. First the various reagents are mixed, and then the following steps are 50 performed to accomplish lyophilization: Individual tubes are placed into a rack/fixture, and the solution is dispensed into each, using existing equipment (e.g., EFD Ultra Dispense Station). The filled rack is placed inside a stainless steel airtight box (modified to accept stoppers in the lid) and then 55 placed into the lyophilization chamber, and the drying cycle commences unattended. During lyophilization, the stoppers are in a raised position allowing air/nitrogen to circulate into, and moisture to exit the stainless box holding racks of vials. At the end of the cycle, the shelves of the lyophilization 60 chamber lower to seat the stoppers into the lid, forming a seal while still inside the closed chamber, in a moisture free nitrogen atmosphere. The steel boxes are then removed from the chamber, and each rack inside is processed in a single operation to seal all vials in that rack. Immediately after sealing, the 65 vials are die cut from the foil in one operation, allowing individual vials to be forwarded to the manufacturer for

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placement into a reagent holder. Internal control can either be added to an existing solution, or can be dispensed into its own cavity in the manner of the collection buffer, wash, neutralization, and release solutions. If lyophilization is required, it will be accomplished in the same manner as the PCR chemistry, and later snapped into the strip. Shelf life stability studies are underway.

Real-Time PCR

After all the appropriate PCR lanes of the PCR cartridge are loaded with final PCR solution, the tray containing the cartridge moves it in the PCR Analyzer. The cartridge is pressed by the optical detection read-head against the heater. Heaters activate valves to close either ends of the PCR reactor and real-time thermocycling process starts. After completing appropriate PCR cycles (~45 cycles), the analyzer decides whether the sample has the target DNA based on the output fluorescence data.

#### Example 3

#### **Exemplary Diagnostic Apparatus**

The apparatus, in combination with the associated con-25 sumables (reagents, cartridges, etc.), automatically performs all aspects of nucleic acid testing, including sample preparation, amplification, and detection for up to 48 samples per hour with the first 24 results available in less than an hour. The system is straightforward to use. An operator simply aliquots a portion of the patient sample into a dedicated tube that contains pre-packaged buffer. The operator places the dedicated tubes into positions on a sample rack. The operator then loads a disposable plastic reagent strip for the appropriate test in the rack. The only other consumable used in the apparatus are microfluidic PCR cartridges for conducting amplification and detection; each cartridge is capable of performing up to twelve PCR tests and two cartridges can be loaded into the analyzer at once. Should the apparatus require a new PCR cartridge, the apparatus will prompt the operator to load the cartridge. The apparatus will then prompt the operator to close the lid to initiate testing. All consumables and sample tubes are barcoded for positive sample identification.

Sample lysis and DNA preparation, which will require approximately half an hour for a full run of 24 samples, is automatically performed by the analyzer's robotic and liquid handling components using protocols and reagents located in unitized, disposable plastic strips. The apparatus then automatically mixes the sample and PCR reagents, and injects the mixture into a cartridge that will be automatically processed by an integrated PCR machine. Rapid, real time PCR and detection requires less than 20 minutes. Results, which will be automatically available upon completion of PCR, are displayed on the instrument's touch screen, printed or sent to the hospital information system, as specified by the user (or the user's supervisor).

Each instrument can process up to 24 samples at a time with a total throughput of 48 samples per hour after the first run. The apparatus is slightly less than 1 m wide and fits easily on a standard lab bench. All operations of the unit can be directed using the included barcode wand and touch screen. The analyzer can be interfaced with lab information systems, hospital networks, PCs, printers or keyboards through four USB interfaces and an Ethernet port.

The apparatus has the following characteristics.

Sensitivity: the apparatus will have a limit of detection of ~50 copies of DNA or RNA. (and may have a limit of detection as low as 25-30 copies of DNA/RNA).

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Cost per Test: Due to the miniaturized, simplified nature of the reagents described herein and also in U.S. patent application Ser. No. 12/172,208, filed Jul. 11, 2008 and incorporated herein by reference, cartridge, and other consumables, the cost of goods per test will be relatively low and very competi-

Automation: By contrast with current "automated" NAT systems, which all require some degree of reasonably extensive technologist interaction with the system, through the use of unitized tests and full integration of sample extraction, 10 preparation, amplification and detection, the apparatus herein will offer a higher level of automation, and corresponding reduction in technologist time and required skill level, thereby favorably impacting overall labor costs.

Throughput: Throughput is defined as how many tests a 15 system can conduct in a given amount of time. The apparatus will be capable of running 45 tests per hour, on average.

Time to First Result: In a hospital environment, time to first result is an especially important consideration. The apparatus will produce the first 24 results in less than an hour and an 20 additional 24 results every half hour thereafter.

Random Access and STAT: Random access is the ability to run a variety of tests together in a single run and place samples in unassigned locations on the analyzer. Also, with chemistry and immunoassay systems, it is desirable to be able to add 25 tests after a run has started. This is often referred to as "true random access" since the user is provided complete flexibility with regard to what tests can be run where on an analyzer and when a new sample can be added to a run. A STAT is a sample that requires as rapid a result as possible, and therefore is 30 given priority in the testing cue on the analyzer. Today, essentially all chemistry and immunoassay analyzers are true random access and offer STAT capabilities. For NAT, however, very few systems offer any random access or STAT capabilities. The instrument herein will provide random access and 35 STAT capabilities.

Menu: The number and type of tests available for the analyzer is a very important factor in choosing systems. The apparatus herein deploys a launch menu strategy that involves a mix of high volume, "standard" nucleic acid tests combined 40 with novel, high value tests.

The apparatus enables 24 clinical samples to be automatically processed to purify nucleic acid, mix the purified DNA/ RNA with PCR reagents and perform real-time PCR in microfluidic cartridge to provide sample to results in an hour. 45 The exemplary apparatus has two PCR readers, each capable of running a 12 lane microfluidic cartridge using an optical system that has dedicated two-color optical detection system.

The apparatus has the following sub-systems:

clinical samples in unitized disposable strips

Magnetic separator-cum-tube heater assembly (24 heating

A four-probe liquid dispensing head

3-axis gantry to move the pipette head

Two PCR amplification-detection stations, each capable of running PCR in the lanes of a 12-lane microfluidic cartridge, and dedicated 2-color optical detection system for each PCR

Control Electronics

Barcode Reader

Operation: The user gets a work list for each sample, whether they want to detect certain target analyte (such as GBS, Chlamydia, Gonorrhoea, HSV) for each clinical sample. The sample tubes are placed on the rack and for each 65 sample, the user slides in a disposable reagent holder (analyte specific) into a corresponding lane of the rack. The reagent

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holder has all the sample prep reagents, PCR reagents, process tubes as well as disposable pipettes already prepackaged in it. Once all disposables are loaded into the rack, the rack is placed in its location on the instrument. The user then places two 12-lane microfluidic PCR cartridges in the two trays of the PCR reader. The user then closes the door of the instrument and starts the sample processing using the GU1 (Graphical User Interface).

The instrument checks functionality of all subsystems and then reads the barcode of the sample tubes, the unitized reagent disposables and the microfluidic cartridges. Any mismatch with a pre-existing work list is determined and errors are flagged, if necessary. The instrument than goes through a series of liquid processing, heating, magnetic separation to complete the sample preparation steps for the each of the clinical sample, mixes the purified nucleic acid with PCR reagents and dispenses the final mix into a lane of the microfluidic cartridges. After a microfluidic cartridge is loaded with the final PCR mix, the cartridge tray moves and aligns the cartridge in the reader and the optical detection system presses the cartridge against a microfluidic PCR heater surface. On-chip valves are actuated to close the reaction mix and then thermocycling is started to initiate the PCR reaction. At each cycle of PCR (up to 45 cycles), fluorescence from each PCR lane is detected by the optical detection system (2-colors per PCR lane) and final result is determined based on the threshold cycle (Ct).

The sample preparation steps for 24 samples are performed in about 40 minutes and the PCR reaction in about 20 minutes.

Sample Reader

The Reader performs function testing of up to twelve properly prepared patient samples by PCR process (real-time PCR) when used in conjunction with microfluidic (test) cartridges as further described herein. Each unit employs two Reader Modules for a total of up to twenty four tests. (FIGS. 31A and 31B) Operation of the Reader is designed for minimal user interaction, requiring the loading and unloading of test cartridges only. During the "Load Disposables" sequence, the Reader will present a motor actuated tray for installation of the disposable cartridge. Sliding a small knob located in the front of the tray, a spring loaded protective cover will raise allowing the test cartridge to be nested properly in place. The cover is then lowered until the knob selflocks into the tray frame, securing the cartridge and preventing movement during the sample loading sequence.

Once the prepared samples have been dispensed via pipettes into the cartridge, the tray will retract into the Reader, Two sample processing racks, each rack processes up to 12 50 accurately positioning the test cartridge beneath the chassis of the optical assembly. The optical assembly will then be lowered by a captured screw driven stepper motor until contact is made with the test cartridge. At this point the test cartridge is located 1/8" above the target location on the heater assembly. As downward motion continues the test cartridge and its holder within the tray compress springs on the tray frame (these are used later to return the cartridge to it's normal position and able to clear the encapsulated wire bonds located on the heater assembly during tray operation). Movement of 60 the test cartridge and optical assembly is complete once contact with the heater assembly is made and a minimum of 2 psi is obtained across the two-thirds of the cartridge area about the PCR channels and their controlling gates. At this point the testing of the cartridge is performed using the heater assembly, measured with onboard optics, and controlled via software and electronics much in the same manner as currently operated on similar diagnostic instruments.

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Once the functional testing is complete the main motor raises the optic assembly, releasing pressure on the test cartridge to return to its normal position. When commanded, the tray motor operating in a rack-and-pinion manner, presents the tray to the customer for cartridge removal and disposal. When the tray is in the extended position it is suspended above a support block located on the apparatus chassis. This block prevents the cartridge from sliding trough the holder in the tray during loading and acts as a support while samples are pipetted into the disposable cartridge. Also provided in this support block is an assist lever to lift and grasp the disposable cartridge during removal. All components of the tray as well as support block and cartridge lift assist are removable by the customer, without tools, for cleaning and reinstalled easily. Microfluidic PCR Heater Module

The microfluidic PCR heater module comprises a glass wafer with photolithographically defined microheaters and sensors to accurately provide heat for actuation of valves and performing thermocycling required to perform a real-time PCR reaction. The wafer surface has dedicated individually 20 controlled heating zones for each of the PCR lanes in the microfluidic cartridge. For a 12-lane cartridge, there are 12 PCR zones; in the 24-up cartridge, there are 24 PCR heating zones. The individual heaters and sensors are electrically connected to a Printed circuit board using gold or aluminum 25 wire bonds. A thermally compliant encapsulant provides physical protection to the wire bonds. While the present device is made on glass wafer, heaters can be fabricated on Si-on-Glass wafers and other polymeric substrates. Each substrate can provide specific advantages related to its thermal 30 and mechanical properties. Besides using photolithography process, such heating substrates can also be assembled using off-the-shelf electronic components such as power resistors, peltiers, transistors, maintaining the upper heating surface of each of the component to be at the same level to provide 35 heating to a microfluidic cartridge. Temperature calibration values for each temperature sensor may be stored in EEPROM or other memory devices co-located in the heater PC Board.

#### Example 4

#### Exemplary High-Efficiency Diagnostic Apparatus

A more highly multiplexed embodiment, enables 24 clinical samples to be automatically processed to purify nucleic acids, mix the purified DNA/RNA with PCR reagents and perform real-time PCR in a microfluidic cartridge. This product has a single PCR reader, with a scanning read-head, capable of reading up to 4 different colors from each of the 50 PCR lanes in a microfluidic cartridge. The cartridge has 24 PCR channels enabling a single cartridge to run all 24 clinical samples. In addition, this apparatus includes a cartridge autoloader, whereby the instrument automatically feeds the PCR reader from a pack of cartridges and discards each used cartridge into a waste tray.

The apparatus has the same sub-systems as the apparatus of Example 3, herein, except in the following: a single PCR amplification-detection station capable of running a 24-lane microfluidic cartridge is used in place of two such stations 60 each running 12-lane cartridges; a scanner unit can detect up to 4 colors from each PCR lane in place of a 2-color optical detection system; and an autoloader unit to feed 24-lane microfluidic cartridge from a box into the PCR detection unit can be used in place of manual loading of a cartridge.

Operation of the instrument is similar to that of the instrument in Example 3, herein, except that a single 24-lane car-

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tridge is loaded, either manually, or from the auto-loader and used cartridge is then pushed out automatically into a waste cartridge bin. Microfluidic cartridges are stored in a cartridge pack (maximum 24 cartridges) and the instrument alerts the user to replace the cartridge pack and empty out the waste cartridge bin once all cartridges from the pack are used up.

#### Example 5

#### Exemplary 24-Lane Cartridge

Various views of an exemplary 24-lane cartridge **5000** are shown in FIGS. **32**A (perspective view), **32**B (plan view), and **32**C (exploded view).

As shown in FIG. 32C, the cartridge 5000 has 3 layers, a laminate 5001, a substrate 5002, and a label 5003. The label is shown in two pieces in this example. A barcode 5006 is visible on label 5003. A row of 24 sample inlets 5004 is shown. A ledge 5005 runs along each of the long edges of the cartridge to facilitate placement in a removable receptacle in receiving bay of a diagnostic instrument, or in a cartridge auto-loader, as further described elsewhere herein.

FIG. 32B has three plan views of the cartridge substrate (upper surface, edge, and lower surface), and two inset views. The upper inset view ("Detail A") shows dimensions of a cut-out (notch) in the upper edge of the cartridge. The right inset view ("Detail B") shows a pair of adjacent lanes, each having a microfluidic network, and each from one of the two banks of networks. The chambers in adjacent networks (from alternate banks) are staggered with respect to one another, even though the sample inlets are all shown in a single line.

The 24-lane cartridge has two banks of 12 PCR lanes, shown in plan view in FIG. 32B. Each lane has a liquid inlet port, that interfaces with a disposable pipette tip; a 4 micro-liter PCR reaction chamber labeled 3A in the inset in FIG. 32B (1.5 mm wide, 300 microns deep, and approximately 10 mm long), two microvalves on either side of the PCR reactor (one of which labeled 3B in the inset in FIG. 32B, and an outlet vent. Microvalves are normally open initially and close the channel on actuation. The outlet holes enable extra liquid (~1 µl) to be contained in the fluidic channel in case more than 6 µl of fluid is dispensed into a lane of the cartridge.

Dimensions of the cartridge (in inches) and layout of the lanes in the cartridge are shown in FIG. 32B. It would be understood that these dimensions and layout are exemplary, and deviations from those shown are consistent with an equivalent manner of operation of such a cartridge.

The inlet holes **5004** of the cartridge are made conical in shape and have a diameter of 3-6 mm at the top to ensure pipettes can be easily landed by the fluid dispensing head within the conical hole. The bigger the holes, the better is the alignment with the pipette, however, there is a trade-off between maximizing the number of inlet ports within the width of the cartridge and ensuring that the pitch between the holes is compatible with the inter-pipette distance. In this particular design, the inter-pipette distance is 24 mm and the distance between the loading holes in the cartridge is 8 mm. So lanes 1, 4, 7, 11 are pipetted into during one dispensing operation; lanes 2, 5, 8 and 12 in the next, and so on and so forth.

The height of the conical holes is kept lower than the height of the ledges on the sides of the cartridge to ensure the cartridges can be stacked on the ledges. The ledges on the two long edges of the cartridge enable stacking of the cartridges with minimal surface contact between two stacked cartridges and also help guide the cartridge into the reader from a cartridge pack in an auto-loader.

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The foregoing description is intended to illustrate various aspects of the present inventions. It is not intended that the examples presented herein limit the scope of the present inventions. The technology now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed is:

- 1. A method of carrying out amplification independently on a plurality of polynucleotide-containing samples, the method comprising:
  - introducing the plurality of samples separately into a microfluidic cartridge;
  - isolating the samples in the microfluidic cartridge;
  - placing the microfluidic cartridge in thermal communication with an array of independent heaters; and
  - amplifying polynucleotides in the plurality of samples by independent application of successive temperature cycles to each sample.
- 2. The method of claim 1, wherein the cartridge contains a plurality of reaction chambers.
- 3. The method of claim 2, wherein the reaction chambers are configured to permit thermal cycling of the plurality of samples independently of one another.
- **4**. The method of claim **2**, wherein isolating the samples in the microfluidic cartridge comprises isolating the samples in the plurality of reaction chambers.
- 5. The method of claim 4, wherein isolating the samples in the plurality of reaction chambers comprises moving the plurality of samples independently of one another into the respective plurality of reaction chambers.
- **6**. The method of claim **5**, wherein isolating the samples in the plurality of reaction chambers further comprises:
  - moving the plurality of samples independently of one another into the respective plurality of reaction chambers:
  - detecting the presence of the plurality of samples in the reaction chambers; and
  - closing a valve on the downstream side of the reaction chamber and closing a valve on the upstream side of the reaction chambers.
- 7. The method of claim 6, wherein detecting the presence of the plurality of samples in the reaction chambers comprises positioning a LED and photodiode in optical communication with reaction chambers.

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- 8. The method of claim 1, wherein amplifying polynucleotides in the plurality of samples comprises independently activating one or more heaters in independent thermal communication with each sample.
- 9. The method of claim 1, wherein introducing the plurality of samples separately into a microfluidic cartridge comprises: placing a plurality of pipettes containing the samples into a plurality of inlets in the microfluidic cartridge; and dispensing the samples independently from the plurality of pipettes into separate of said plurality of inlets.
- 10. The method of claim 1, wherein the plurality of samples are introduced into the microfluidic cartridge simultaneously.
- 11. The method of claim 1, wherein the plurality of samples are introduced into the microfluidic sample successively.
- 12. The method of claim 1, further comprising detecting the presence of amplified polynucleotides in the plurality of samples.
- 13. The method of claim 12, wherein detecting the presence of amplified polynucleotides comprises detecting a fluorescence signal from the amplified polynucleotides.
- 14. The method of claim 13, wherein detecting a fluorescence signal from the amplified polynucleotides comprises passing a scanning read head over the microfluidic cartridge, the scanning read head comprising a plurality of detectors having a LED and photodiode.
- **15**. A method of carrying out amplification independently on a plurality of polynucleotide-containing samples, the method comprising:
  - introducing the plurality of samples in to a microfluidic cartridge, wherein the cartridge has a plurality of reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another;
  - moving the plurality of samples independently of one another into the respective plurality of reaction chambers;
  - isolating the samples within the plurality of reaction chambers;
  - placing the microfluidic cartridge in thermal communication with an array of independent heaters; and
  - amplifying polynucleotides contained within the plurality of samples, by application of successive temperature cycles independently to the reaction chambers.

\* \* \* \* \*

# EXHIBIT 6

## (12) United States Patent Handique

### (10) Patent No.: US 8,709,787 B2 (45) Date of Patent: Apr. 29, 2014

## (54) MICROFLUIDIC CARTRIDGE AND METHOD OF USING SAME

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(US)

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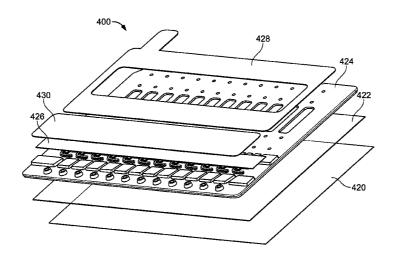
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#### (57) ABSTRACT

The present technology provides for a microfluidic substrate configured to carry out PCR on a number of polynucleotide-containing samples in parallel. The substrate can be a single-layer substrate in a microfluidic cartridge. Also provided are a method of making a microfluidic cartridge comprising such a substrate. Still further disclosed are a microfluidic valve suitable for use in isolating a PCR chamber in a microfluidic substrate, and a method of making such a valve.

#### 22 Claims, 47 Drawing Sheets



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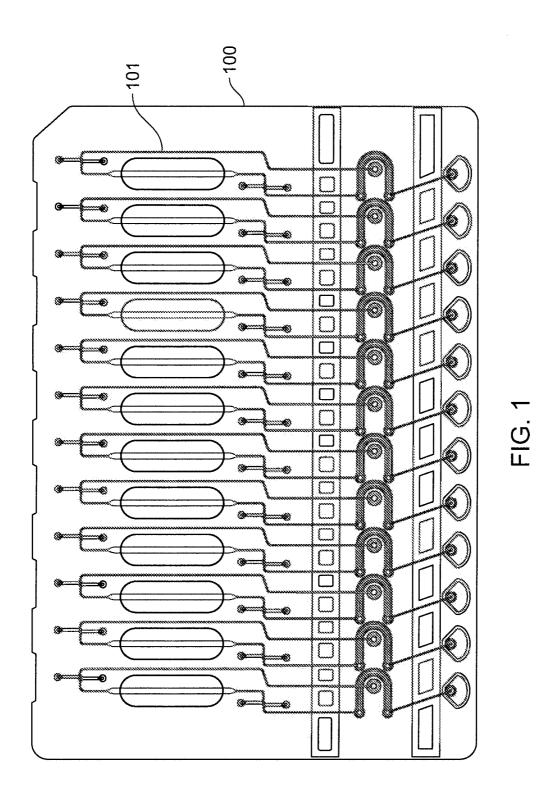
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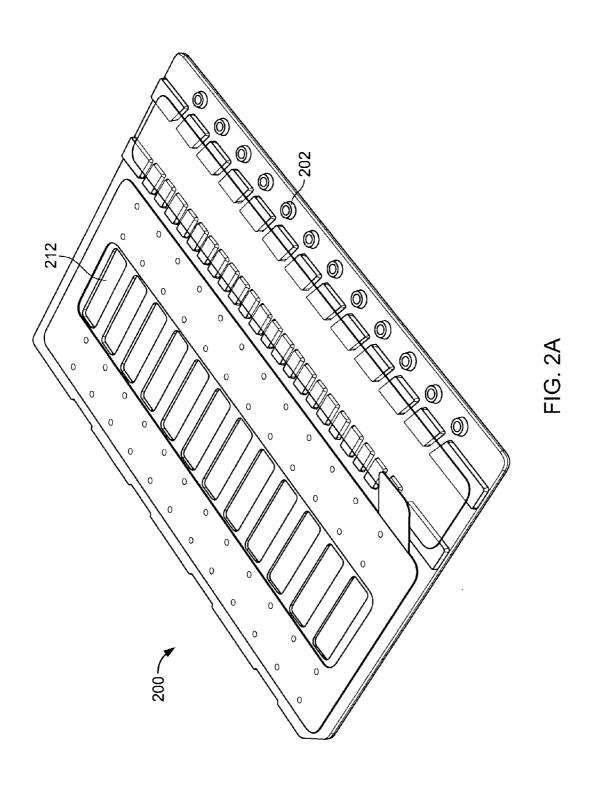
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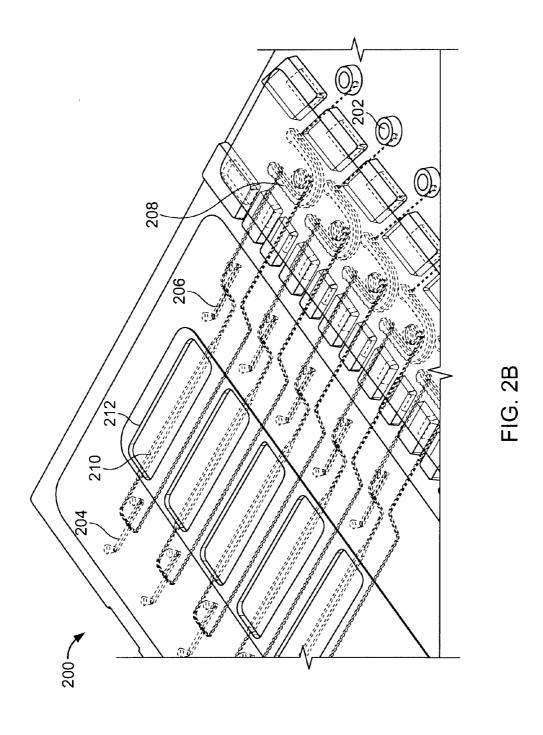
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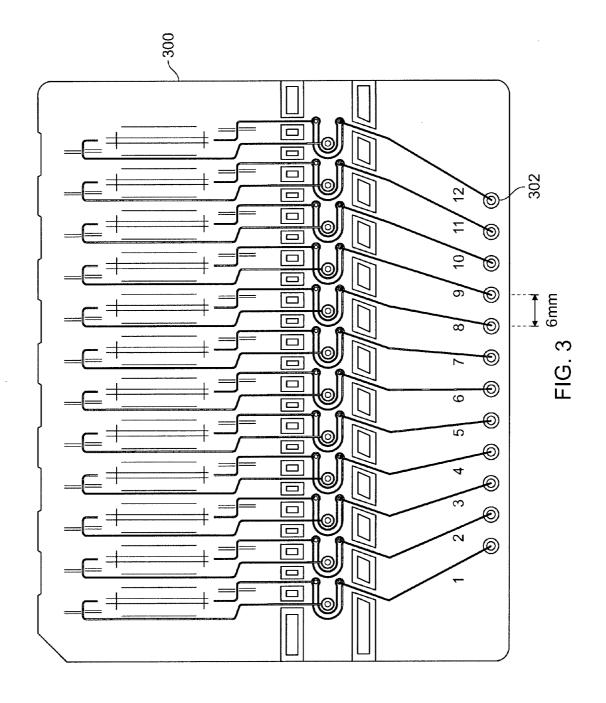
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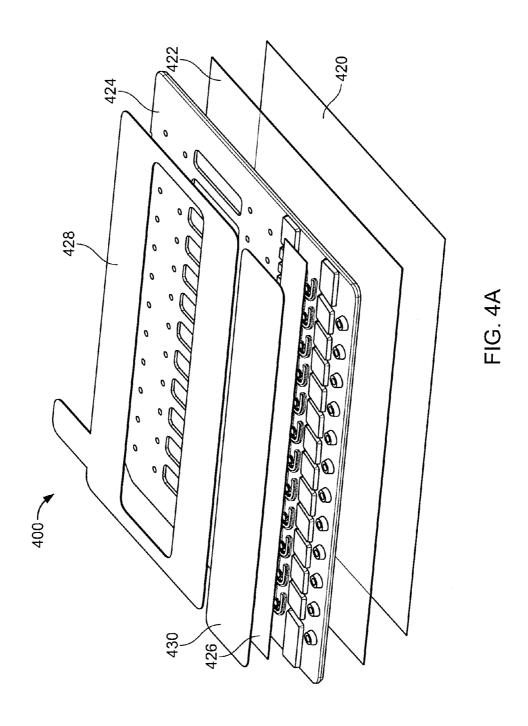
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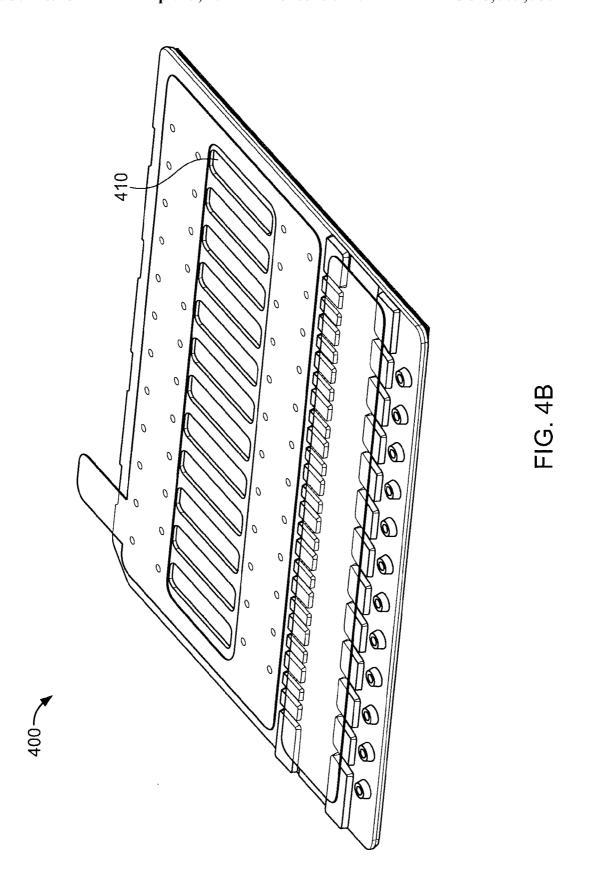


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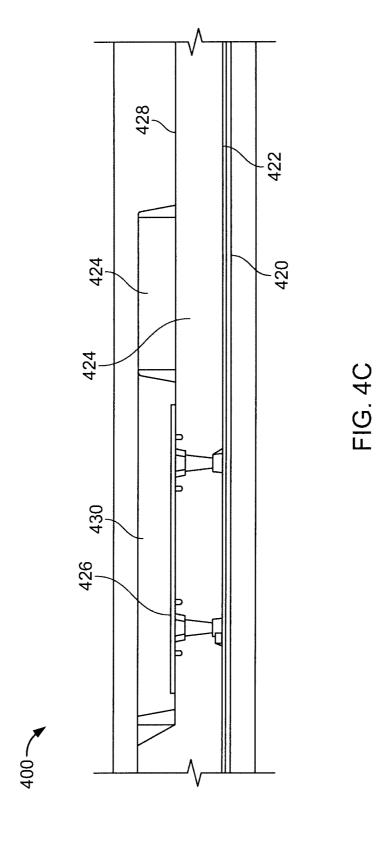
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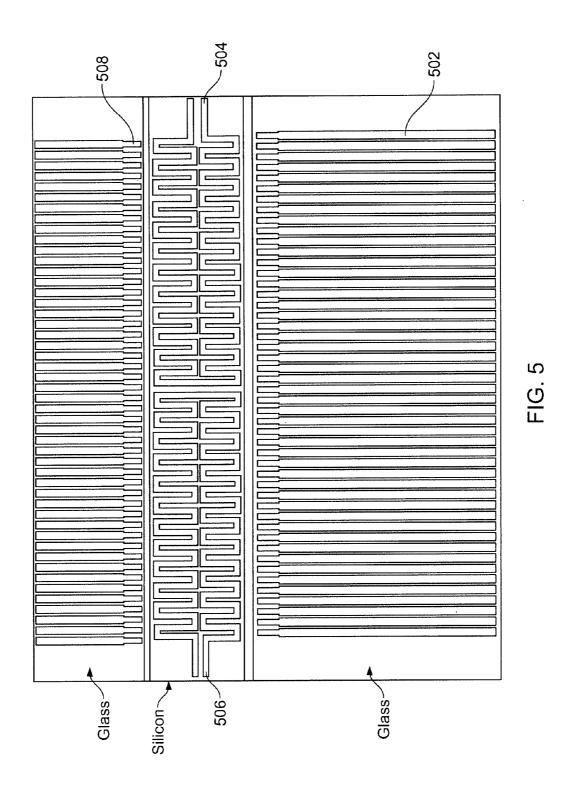
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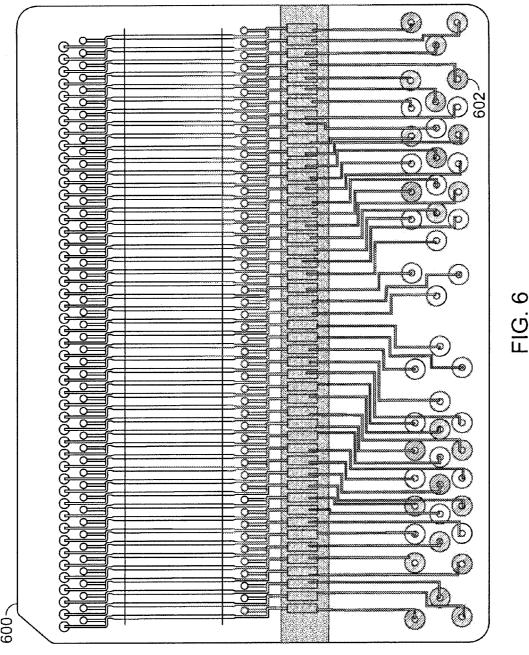
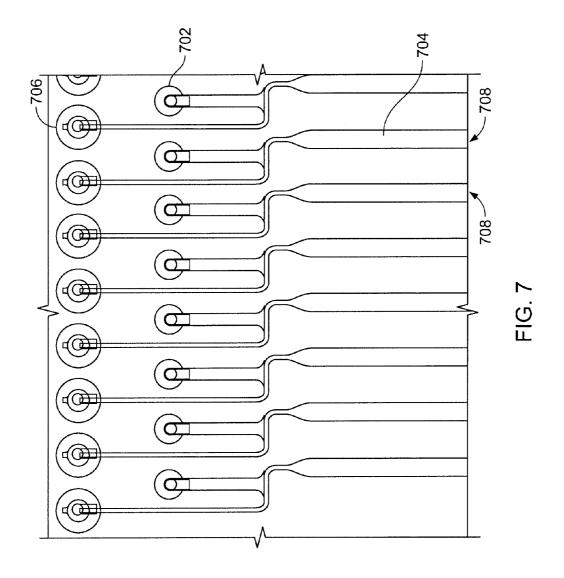


FIG. 6

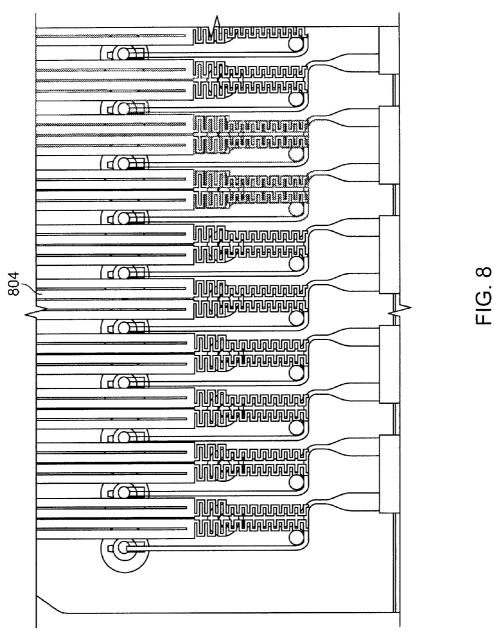
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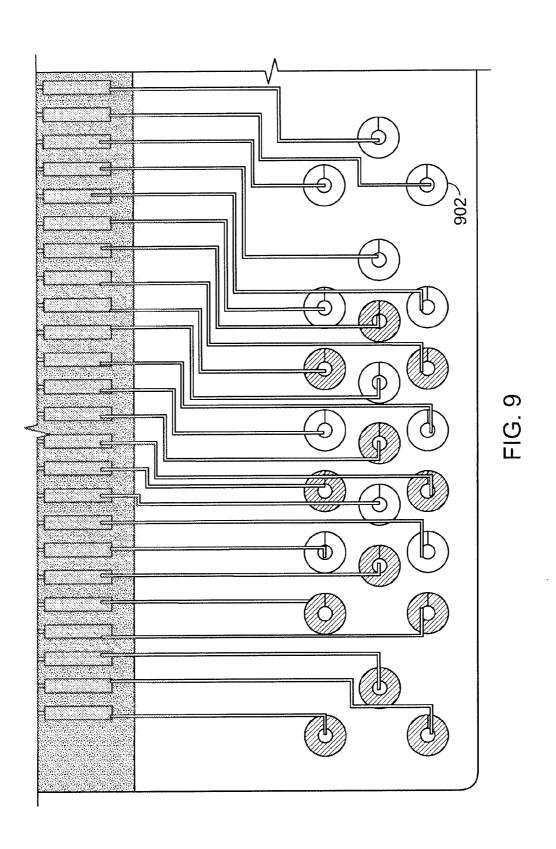
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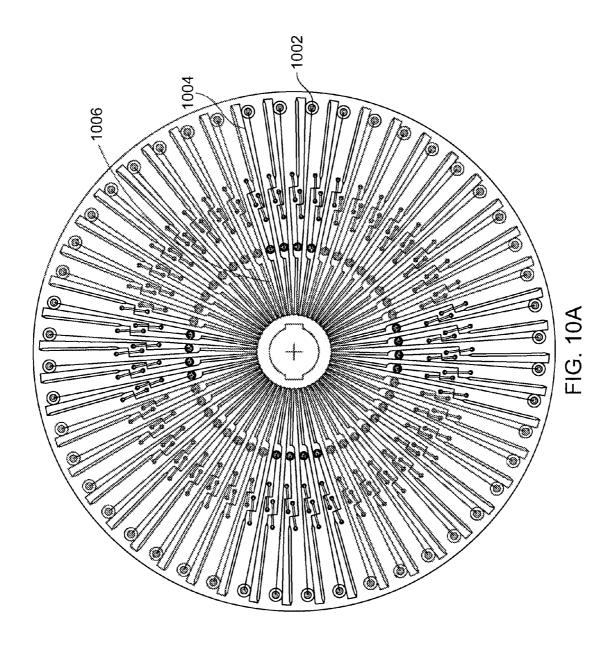
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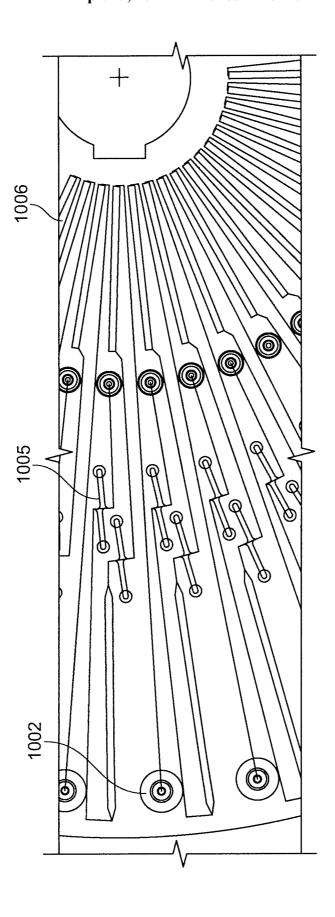
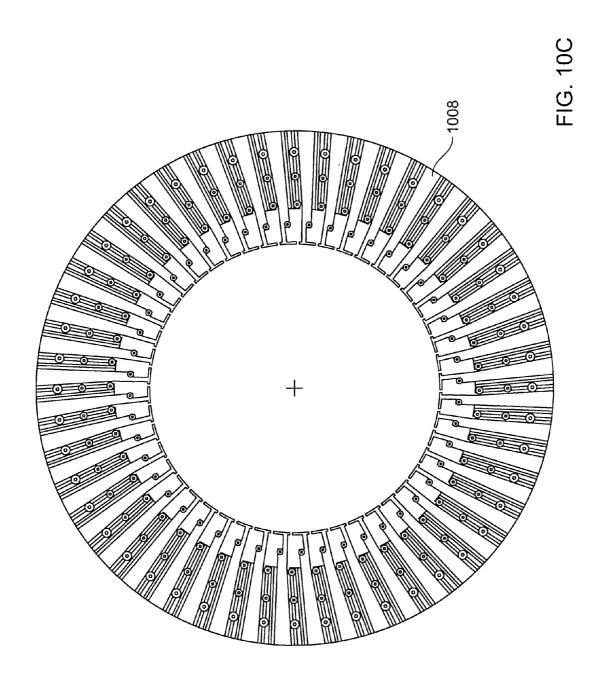


FIG. 10B

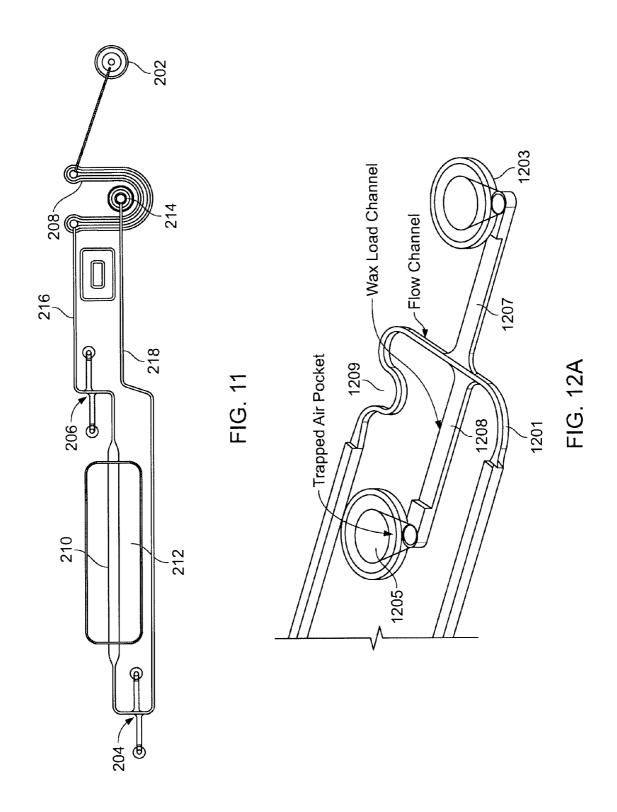
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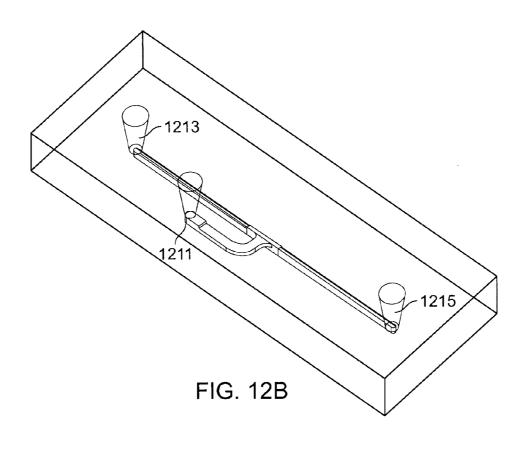
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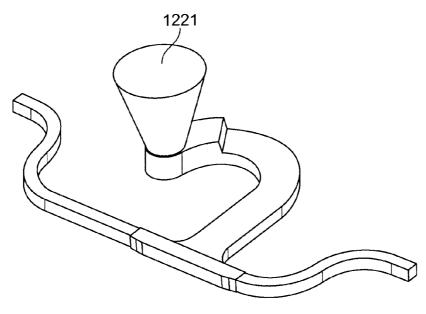
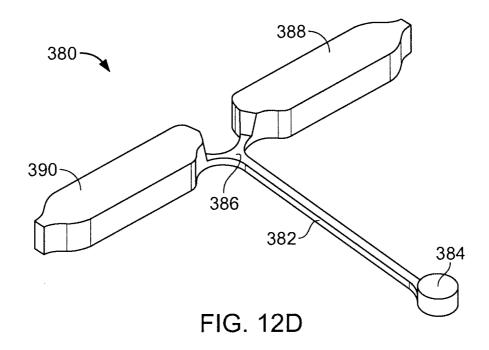


FIG. 12C

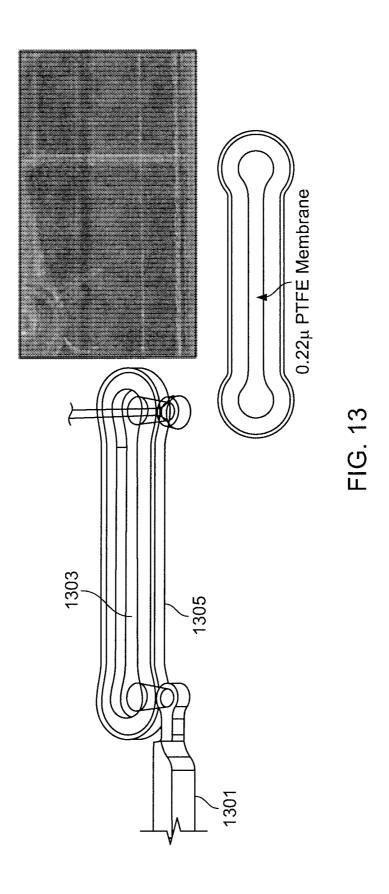
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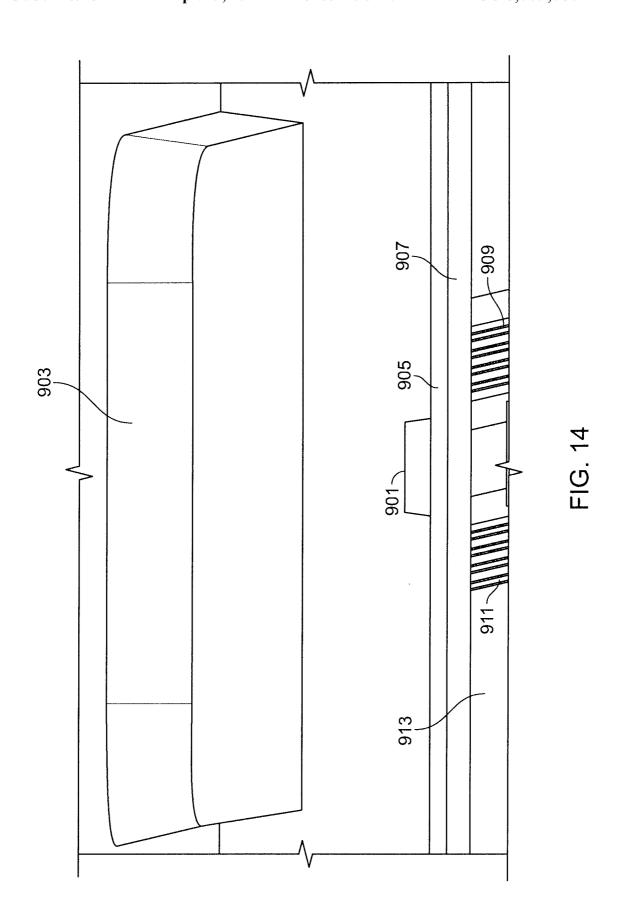
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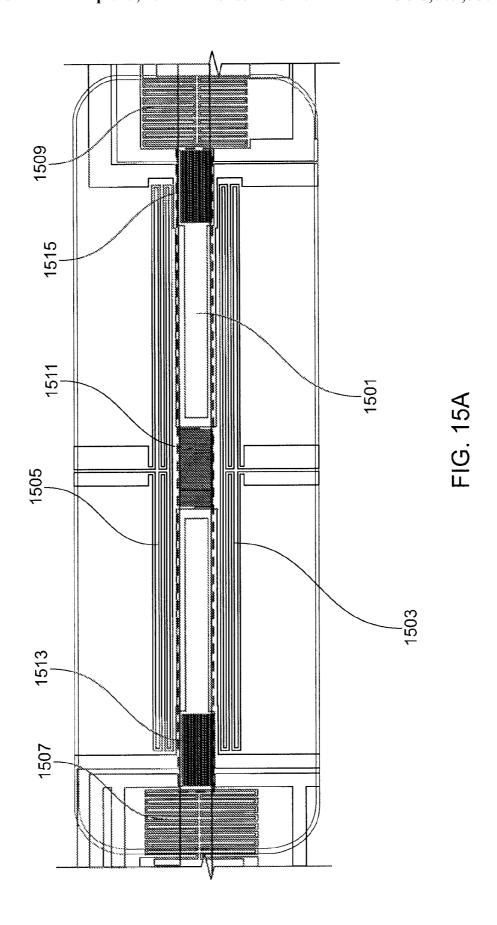
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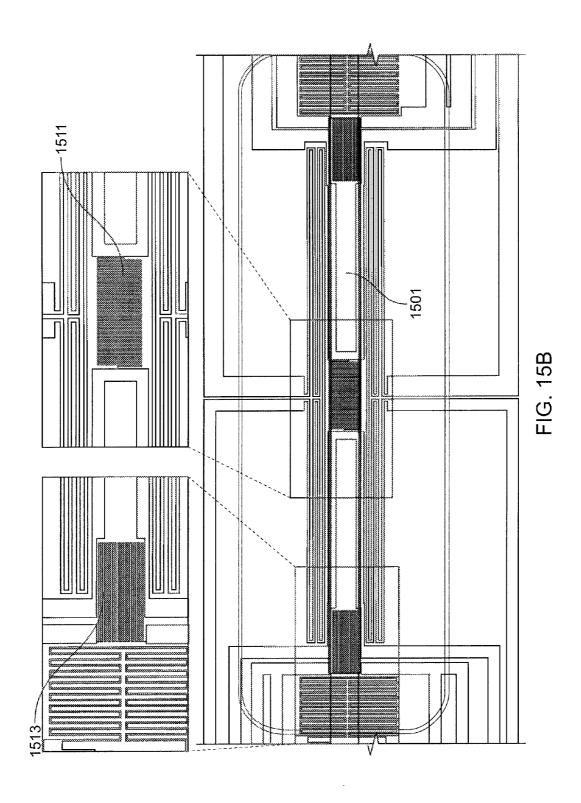
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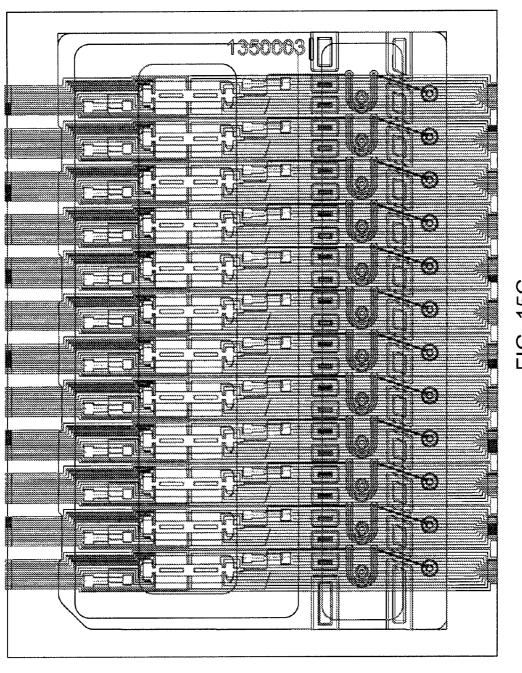
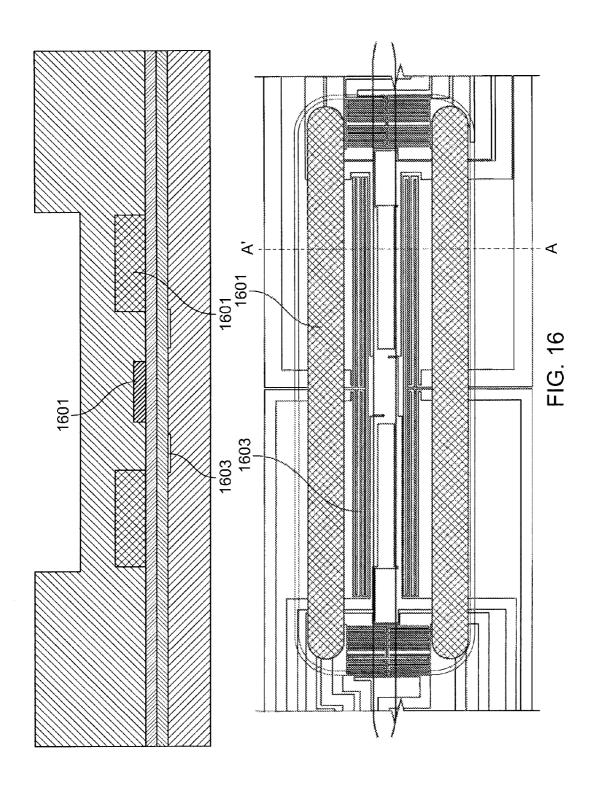


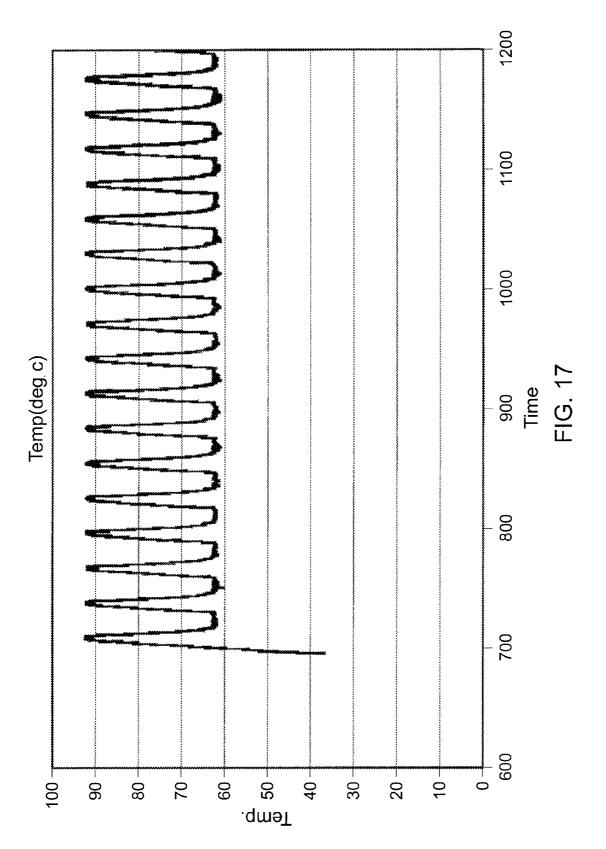
FIG. 15C

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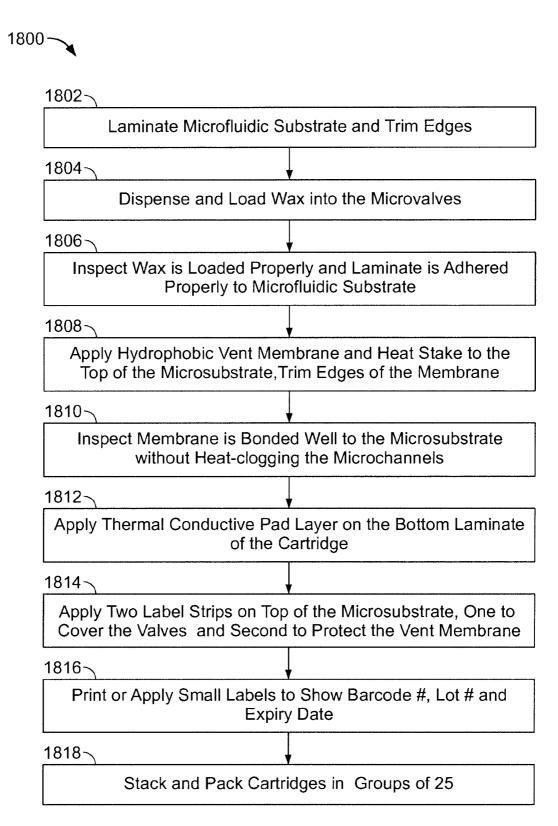


FIG. 18

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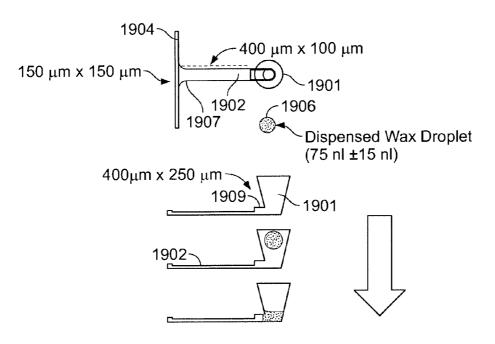
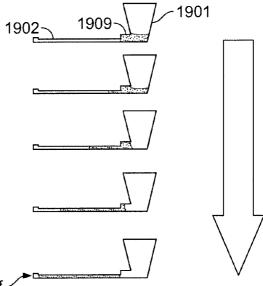


FIG. 19A

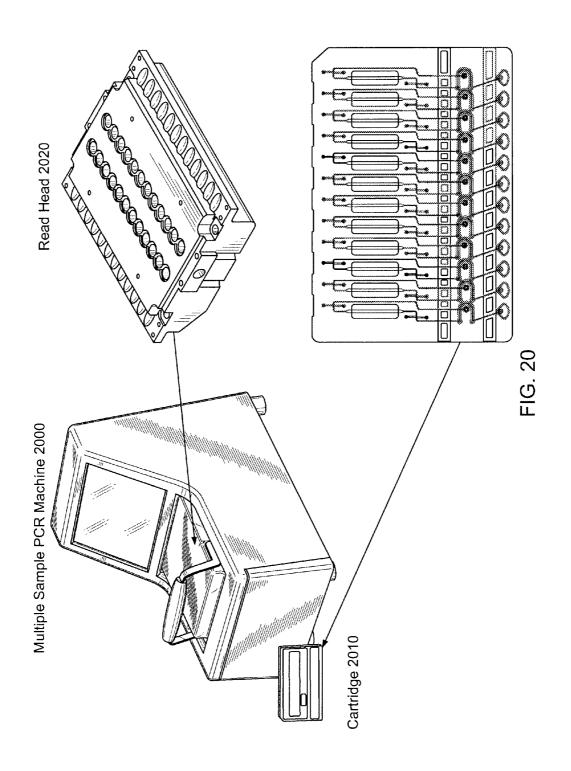


Capillary action of controlled volume of wax causes it to fill up the Wax up to the right interface without blocking the liquid flowable microchannel

FIG. 19B

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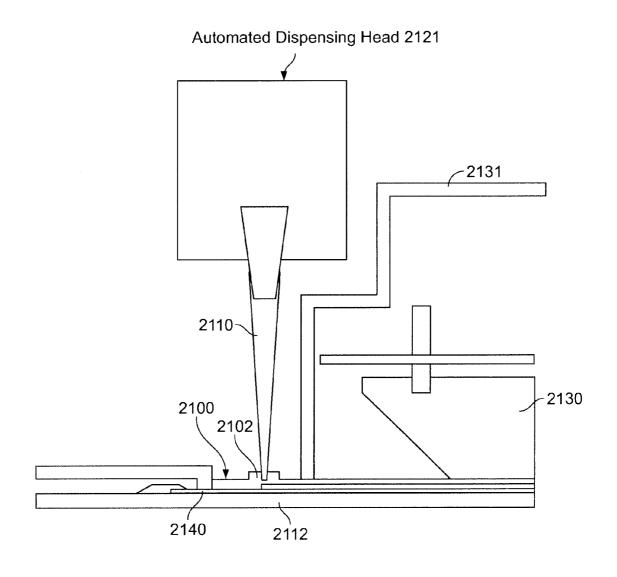


FIG. 21

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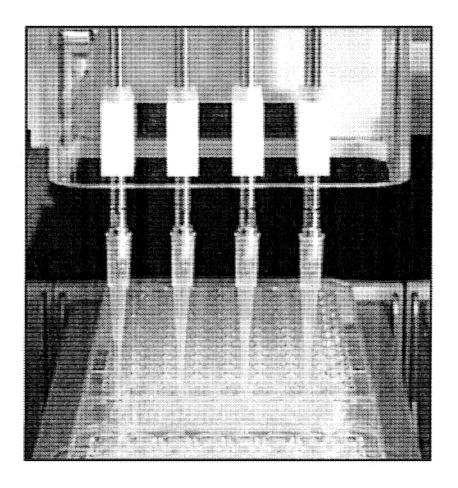
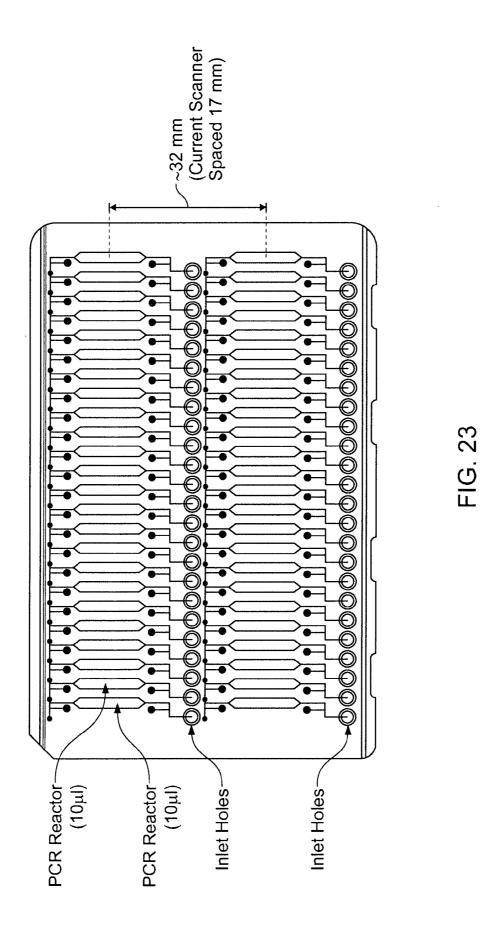


FIG. 22

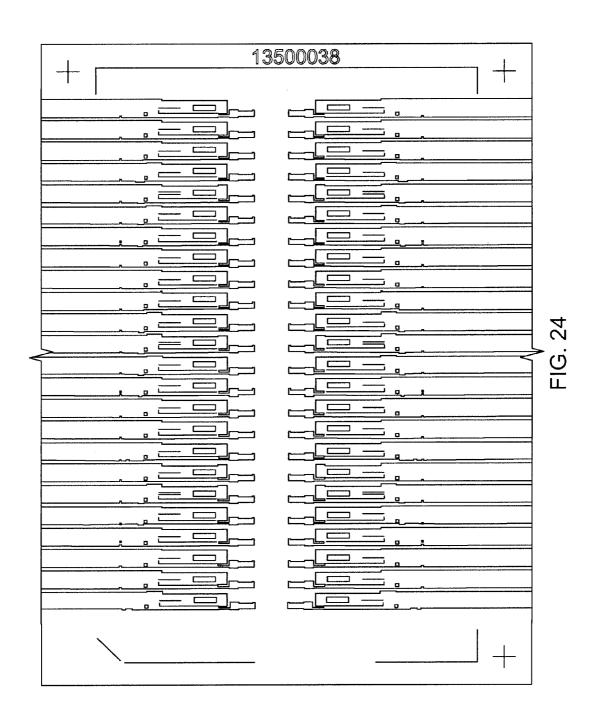
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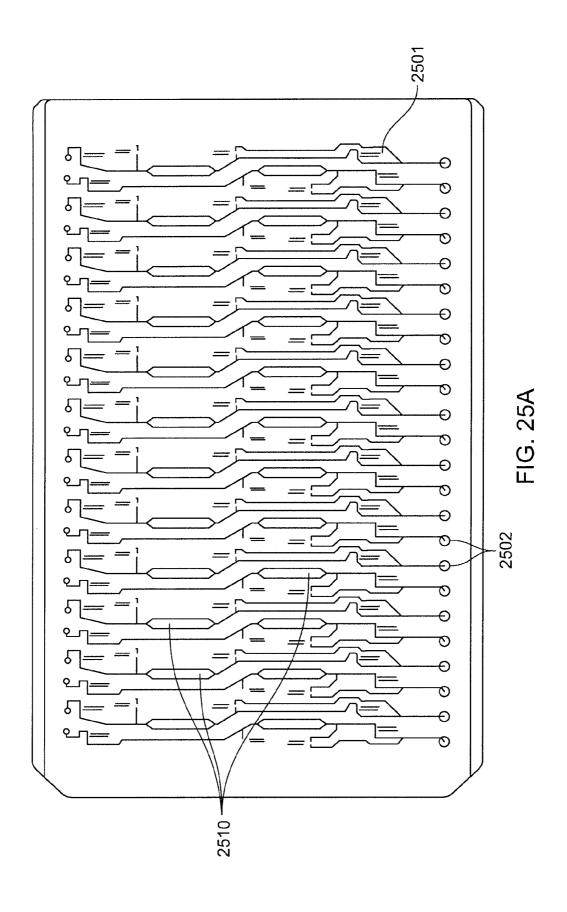
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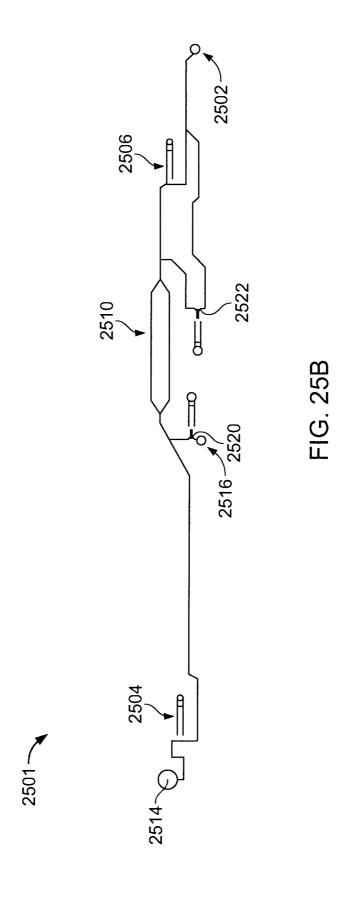
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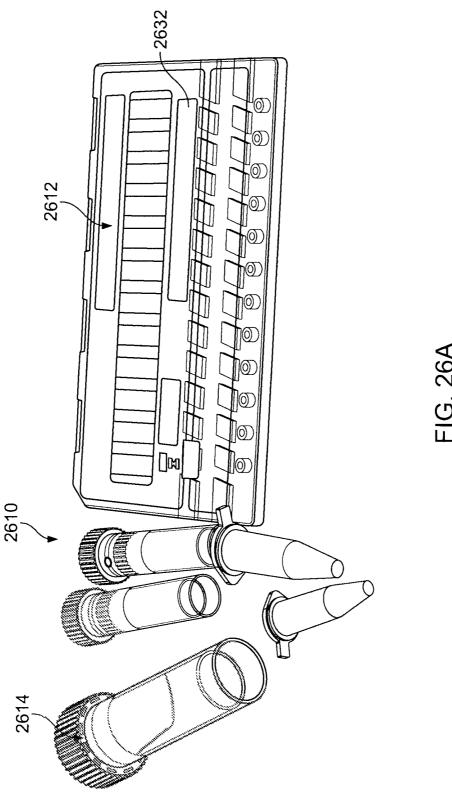
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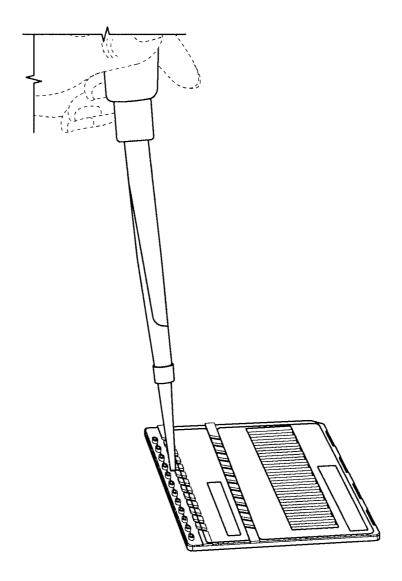


FIG. 26B

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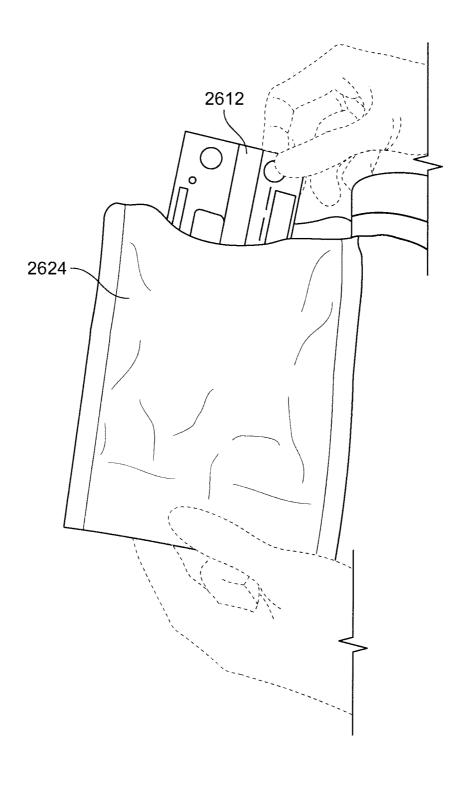


FIG. 27

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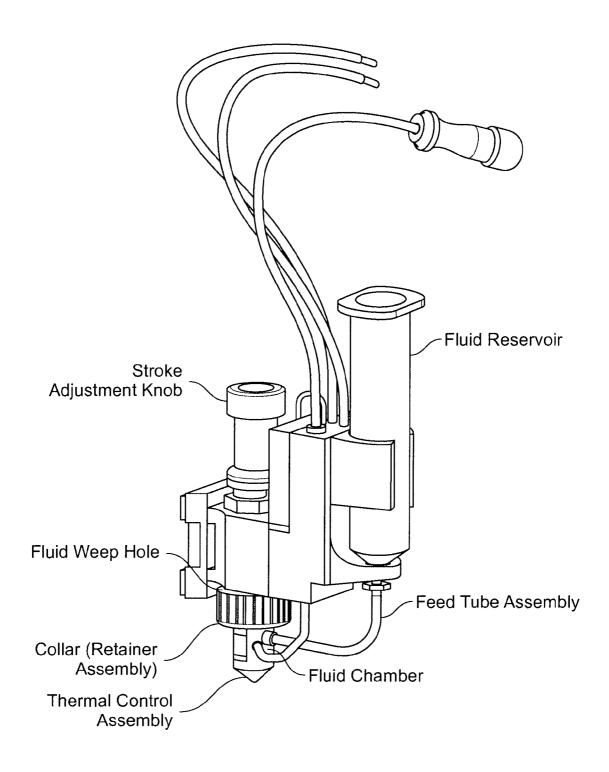


FIG. 28A

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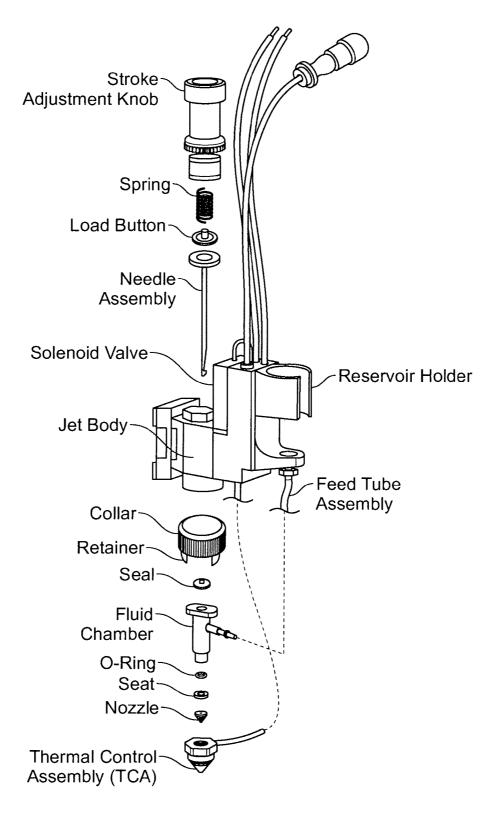
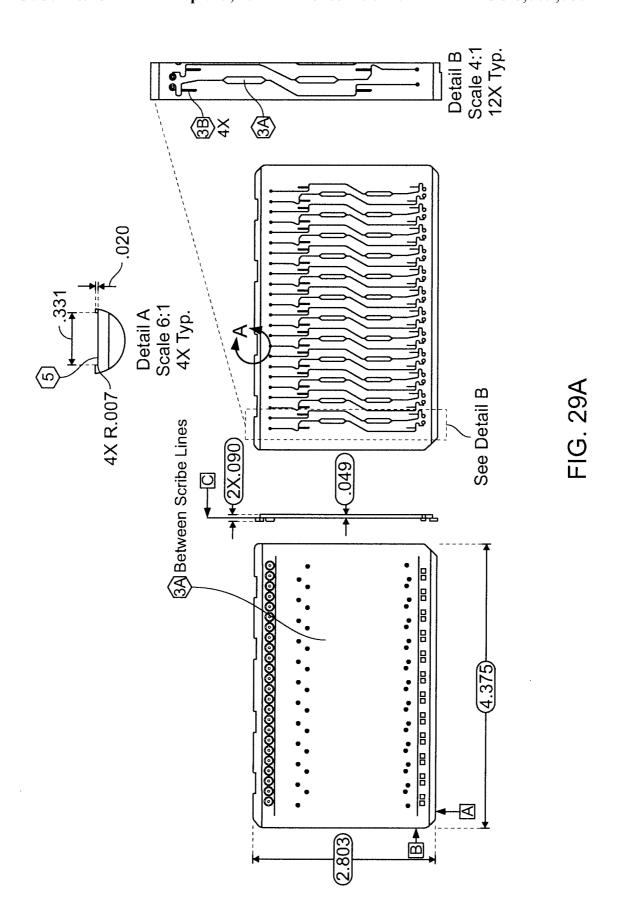


FIG. 28B

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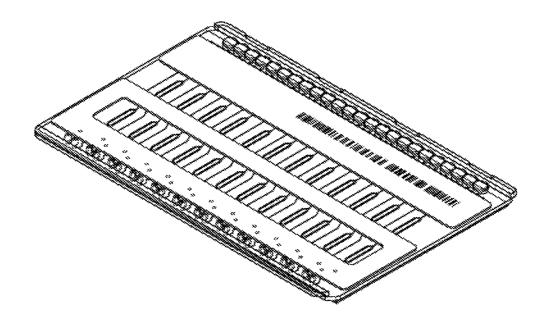


FIG. 29B

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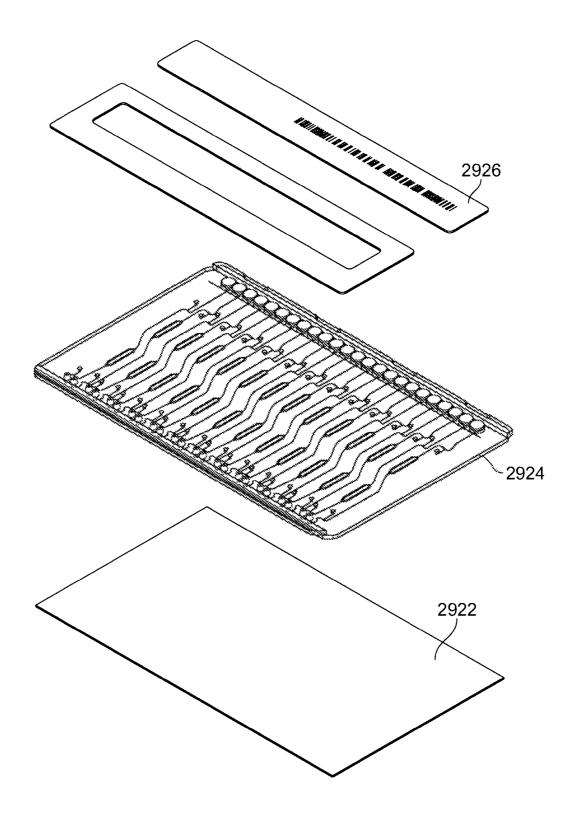


FIG. 29C

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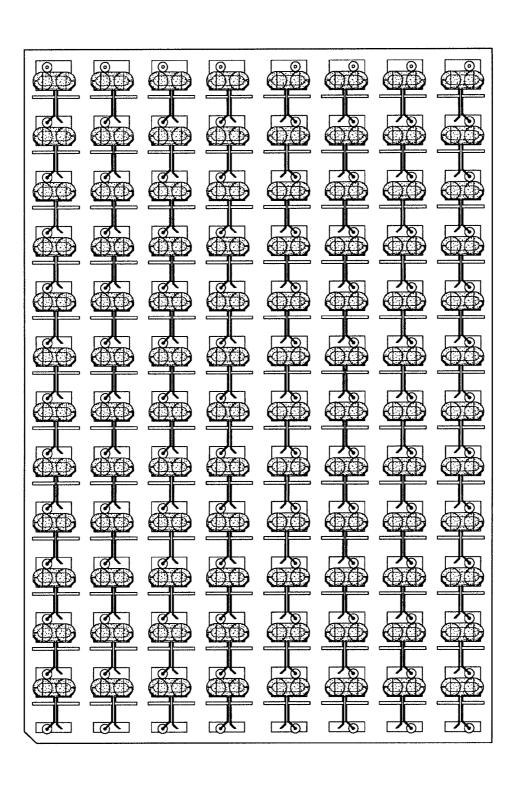


FIG. 30A

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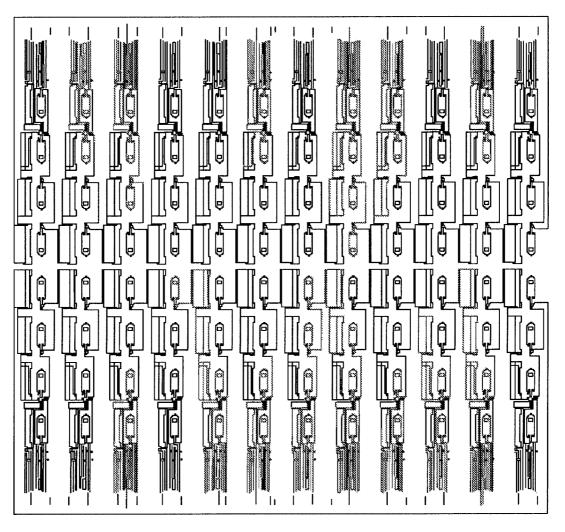


FIG. 30B

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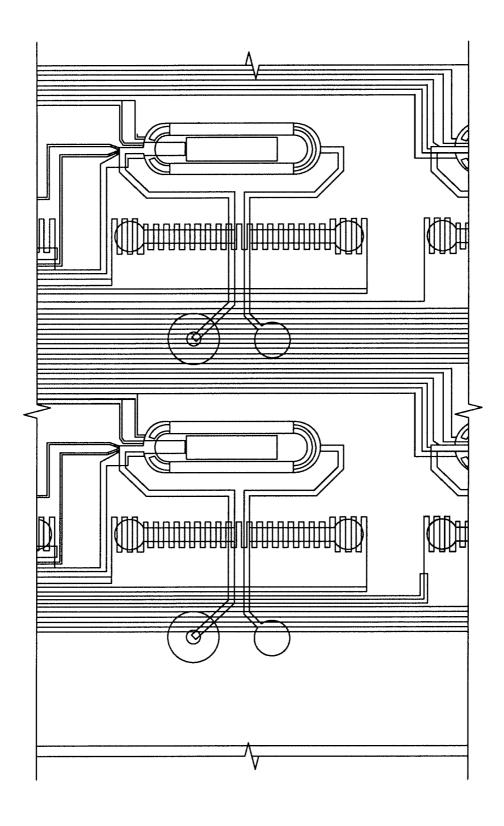
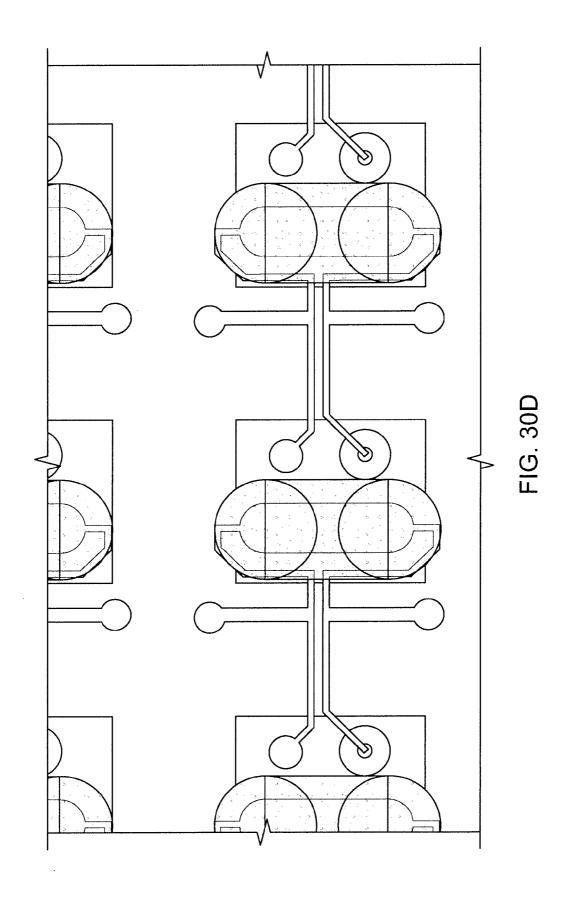


FIG. 30C

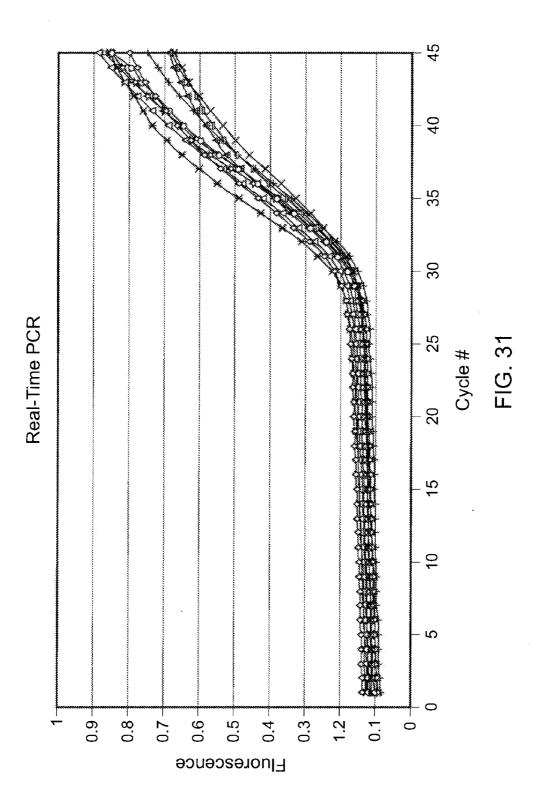
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# MICROFLUIDIC CARTRIDGE AND METHOD OF USING SAME

#### **CLAIM OF PRIORITY**

The instant application claims the benefit of priority to U.S. provisional applications having Ser. Nos. 60/859,284, filed Nov. 14, 2006, and 60/959,437, filed Jul. 13, 2007, the specifications of both of which are incorporated herein by reference in their entireties.

#### TECHNICAL FIELD

The technology described herein generally relates to microfluidic cartridges. The technology more particularly relates to microfluidic cartridges that are configured to carry out PCR on nucleotides of interest, particularly from several biological samples in parallel, within microfluidic channels in the cartridge, and permit detection of those nucleotides.

#### BACKGROUND

The medical diagnostics industry is a critical element of today's healthcare infrastructure. At present, however, diagnostic analyses no matter how routine have become a bottleneck in patient care. There are several reasons for this. First, many diagnostic analyses can only be done with highly specialist equipment that is both expensive and only operable by trained clinicians. Such equipment is found in only a few locations—often just one in any given urban area. This means that most hospitals are required to send out samples for analyses to these locations, thereby incurring shipping costs and transportation delays, and possibly even sample loss or mixup. Second, the equipment in question is typically not available 'on-demand' but instead runs in batches, thereby delaying the processing time for many samples because they must wait for a machine to fill up before they can be run.

Understanding that sample flow breaks down into several key steps, it would be desirable to consider ways to automate as many of these as possible. For example, a biological sample, once extracted from a patient, must be put in a form suitable for a processing regime that typically involves using polymerase chain reaction (PCR) to amplify a vector of interest. Once amplified, the presence or absence of a nucleotide of interest from the sample needs to be determined unambiguously. Sample preparation is a process that is susceptible to automation but is also relatively routinely carried out in almost any location. By contrast, steps such as PCR and nucleotide detection have customarily only been within the compass of specially trained individuals having access to specialist equipment.

There is therefore a need for a method and apparatus of carrying out PCR on prepared biological samples and detecting amplified nucleotides, preferably with high throughput. In particular there is a need for an easy-to-use device that can deliver a diagnostic result on several samples in a short time.

The discussion of the background to the technology herein is included to explain the context of the technology. This is 55 not to be taken as an admission that any of the material referred to was published, known, or part of the common general knowledge as at the priority date of any of the claims.

Throughout the description and claims of the specification the word "comprise" and variations thereof, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

# SUMMARY

The present technology includes methods and devices for detecting polynucleotides in samples, particularly from bio2

logical samples. In particular, the technology relates to microfluidic devices that carry out PCR on nucleotides of interest within microfluidic channels, and permit detection of those nucleotides.

In particular, the present technology provides for a microfluidic cartridge, comprising: a first PCR reaction chamber; a second PCR reaction chamber; a first inlet, in fluid communication with the first PCR reaction chamber; a second inlet, in fluid communication with the second PCR reaction chamber; a first set of microfluidic valves configured to control motion of a sample from the first inlet to the first PCR reaction chamber; and a second set of microfluidic valves configured to control motion of a sample from the second inlet to the second PCR reaction chamber.

The present technology includes a process for carrying out PCR on a plurality of polynucleotide-containing samples, the method comprising: introducing the plurality of samples into a microfluidic cartridge, wherein the cartridge has a plurality of PCR reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another; moving the plurality of samples into the respective plurality of PCR reaction chambers; and amplifying polynucleotides contained with the plurality of samples, by application of successive heating and cooling cycles to the PCR reaction chambers.

The present technology further comprises a number of other embodiments, as set forth herein.

A microfluidic substrate, comprising: a first PCR reaction chamber; a second PCR reaction chamber; a first inlet, in fluid communication with the first PCR reaction chamber; a second inlet, in fluid communication with the second PCR reaction chamber; a first set of microfluidic valves configured to isolate the first reaction chamber from the first inlet; and a second set of microfluidic valves configured to isolate the second PCR reaction chamber from the second inlet.

A microfluidic substrate, comprising: a plurality of sample lanes, wherein each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another: an inlet; a first valve and a second valve; a first channel leading from the inlet, via the first valve, to a reaction chamber; and a second channel leading from the reaction chamber, via the second valve, to a vent.

A microfluidic cartridge having a plurality of microfluidic networks, wherein each of the microfluidic networks, including a PCR reaction chamber, an inlet hole, and the valves for isolating the PCR reaction chamber, is defined in a single substrate.

A method of carrying out PCR independently on a plurality of polynucleotide-containing samples, the method comprising: introducing the plurality of samples in to a microfluidic cartridge, wherein the cartridge has a plurality of PCR reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another; moving the plurality of samples into the respective plurality of PCR reaction chambers; isolating the plurality of PCR reaction chambers; and amplifying polynucleotides contained with the plurality of samples, by application of successive heating and cooling cycles to the PCR reaction chambers.

A microfluidic valve, comprising: a first chamber, connected to a first load channel; a second chamber, connected to a second load channel; and a flow channel, wherein the first and second load channels are each connected to the flow channel, and wherein the first and second load channels each contain a thermally responsive substance that, upon actuation of the valve, flows into the flow channel thereby sealing it, and wherein the flow channel is constricted along a length either side of the first and second load channels.

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A microfluidic valve, comprising: a chamber, connected to a load channel; and a flow channel, wherein the load channel is connected to the flow channel, and wherein the load channel contains a thermally responsive substance that, upon actuation of the valve, flows into the flow channel thereby sealing it, and wherein the flow channel is constricted along a length either side of the load channel.

A method of making a microfluidic valve, the method comprising: directing a dispensing head over an inlet hole in a microfluidic substrate; propelling a quantity of thermally responsive substance from the dispensing head into the inlet hole; and maintaining a temperature of the microfluidic substrate so that the thermally responsive substance flows by capillary action from the inlet hole into a microfluidic channel in communication with the inlet hole.

The microfluidic cartridge described herein can be configured for use with an apparatus comprising: a chamber configured to receive the microfluidic cartridge; at least one heat source thermally coupled to the cartridge and configured to apply heat and cooling cycles that carry out PCR on one or 20 more microdroplets of polynucleotide-containing sample in the cartridge; a detector configured to detect presence of one or more polynucleotides in the one or more samples; and a processor coupled to the detector and the heat source, configured to control heating of one or more regions of the microfluidic cartridge.

The details of one or more embodiments of the technology are set forth in the accompanying drawings and further description herein. Other features, objects, and advantages of the technology will be apparent from the description and <sup>30</sup> drawings, and from the claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a plan view of an exemplary multi-lane 35 microfluidic cartridge;

FIG. 2A shows an exemplary multi-lane cartridge;

FIG. 2B shows a portion of an exemplary multi-lane cartridge of FIG. 2A;

FIG. 3 shows a plan of microfluidic circuitry and inlets in 40 an exemplary multi-lane cartridge;

FIGS. 4A-4C show layer construction, and cross section of an exemplary microfluidic cartridge;

FIG. 5 shows a heater array for an exemplary highly-multiplexed microfluidic cartridge;

FIGS. **6-9** show various aspects of exemplary highly multiplexed microfluidic cartridges;

FIGS. 10A-10C show various aspects of a radially configured highly multiplexed microfluidic cartridge and associated heater array;

FIG. 11 shows an exemplary microfluidic network in a lane of a multi-lane cartridge such as that for FIG. 1 or 3;

FIGS. 12A-12C show exemplary microfluidic valves, and a gate FIG. 12D;

FIG. 13 shows an exemplary bubble vent;

FIG. 14 shows a cross-section of a portion of a microfluidic cartridge, when in contact with a heater substrate;

FIGS. **15**A and **15**B show a plan view of heater circuitry adjacent to a PCR reaction chamber; FIG. **15**C shows an overlay of an array of heater elements on an exemplary multilane microfluidic cartridge, wherein various microfluidic networks are visible;

FIG. 16 shows various cut-away sections that can be used to improve cooling rates during PCR thermal cycling;

FIG. 17 shows a plot of temperature against time during a 65 PCR process, as performed on a microfluidic cartridge as described herein;

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FIG. 18 shows an exemplary assembly process for a cartridge as further described herein;

FIGS. 19A and 19B show exemplary deposition of wax droplets into microfluidic valves;

FIG. 20 shows an exemplary apparatus, a microfluidic cartridge, and a read head contains a detector, as further described herein;

FIG. 21 shows a cross-section of a pipetting head and a cartridge in position in a microfluidic apparatus;

FIG. **22** shows introduction of a PCR-ready sample into a cartridge, situated in an instrument;

FIG. 23 shows an exemplary 48-lane cartridge;

FIG. **24** shows a heater configuration used for actuating the 48-lane PCR cartridge of FIG. **23**;

FIGS. 25A and 25B respectively show an exemplary cartridge and lane configuration for a cartridge that permits retrieval of amplified sample;

FIG. **26**A shows components of a kit, including an exemplary cartridge and reagents; FIG. **26**B shows manual action of pipetting a PCR solution into PCR lanes of the cartridge;

FIG. 27 shows an exemplary cartridge partially removed from a sealed pouch;

FIGS. 28A and 28B show exemplary apparatus for carrying out wax deposition;

FIGS. **29**A-**29**C show an exemplary 24-lane cartridge in plan view, perspective views, and cross-section, respectively; FIGS. **30**A-**30**D show aspects of a 96-lane cartridge; and FIG. **31** shows a real-time PCR trace.

#### DETAILED DESCRIPTION

Microfluidic Cartridge

The present technology comprises a microfluidic cartridge that is configured to carry out an amplification, such as by PCR, of one or more polynucleotides from one or more samples. It is to be understood that, unless specifically made clear to the contrary, where the term PCR is used herein, any variant of PCR including but not limited to real-time and quantitative, and any other form of polynucleotide amplification is intended to be encompassed. The microfluidic cartridge need not be self-contained and can be designed so that it receives thermal energy from one or more heating elements present in an external apparatus with which the cartridge is in thermal communication. An exemplary such apparatus is further described herein; additional embodiments of such a system are found in U.S. patent application Ser. No. 11/985,577, entitled "Microfluidic System for Amplifying and Detecting Polynucleotides in Parallel", and filed on even date herewith, the specification of which is incorporated herein by reference.

By cartridge is meant a unit that may be disposable, or reusable in whole or in part, and that is configured to be used in conjunction with some other apparatus that has been suitably and complementarily configured to receive and operate on (such as deliver energy to) the cartridge.

By microfluidic, as used herein, is meant that volumes of sample, and/or reagent, and/or amplified polynucleotide are from about 0.1  $\mu$ l to about 999  $\mu$ l, such as from 1-100  $\mu$ l, or from 2-25  $\mu$ l. Similarly, as applied to a cartridge, the term microfluidic means that various components and channels of the cartridge, as further described herein, are configured to accept, and/or retain, and/or facilitate passage of microfluidic volumes of sample, reagent, or amplified polynucleotide. Certain embodiments herein can also function with nanoliter volumes (in the range of 10-500 nanoliters, such as 100 nanoliters).

One aspect of the present technology relates to a microfluidic cartridge having two or more sample lanes arranged so

that analyses can be carried out in two or more of the lanes in parallel, for example simultaneously, and wherein each lane is independently associated with a given sample.

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A sample lane is an independently controllable set of elements by which a sample can be analyzed, according to 5 methods described herein as well as others known in the art. A sample lane comprises at least a sample inlet, and a microfluidic network having one or more microfluidic components, as further described herein.

In various embodiments, a sample lane can include a sample inlet port or valve, and a microfluidic network that comprises, in fluidic communication one or more components selected from the group consisting of: at least one thermally actuated valve, a bubble removal vent, at least one thermally actuated pump, a gate, mixing channel, positioning 15 element, microreactor, a downstream thermally actuated valve, and a PCR reaction chamber. The sample inlet valve can be configured to accept a sample at a pressure differential compared to ambient pressure of between about 70 and 100 kilopascals.

The cartridge can therefore include a plurality of microfluidic networks, each network having various components, and each network configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined.

A multi-lane cartridge is configured to accept a number of samples in series or in parallel, simultaneously or consecutively, in particular embodiments 12 samples, wherein the samples include at least a first sample and a second sample, wherein the first sample and the second sample each contain 30 one or more polynucleotides in a form suitable for amplification. The polynucleotides in question may be the same as, or different from one another, in different samples and hence in different lanes of the cartridge. The cartridge typically processes each sample by increasing the concentration of a polynucleotide to be determined and/or by reducing the concentration of inhibitors relative to the concentration of polynucleotide to be determined.

The multi-lane cartridge comprises at least a first sample lane having a first microfluidic network and a second lane 40 having a second microfluidic network, wherein each of the first microfluidic network and the second microfluidic network is as elsewhere described herein, and wherein the first microfluidic network is configured to amplify polynucleotides in the first sample, and wherein the second microfluidic 45 network is configured to amplify polynucleotides in the second sample.

In various embodiments, the microfluidic network can be configured to couple heat from an external heat source to a sample mixture comprising PCR reagent and neutralized 50 polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

At least the external heat source may operate under control of a computer processor, configured to execute computer 55 readable instructions for operating one or more components of each sample lane, independently of one another, and for receiving signals from a detector that measures fluorescence from one or more of the PCR reaction chambers.

For example, FIG. 1 shows a plan view of a microfluidic 60 cartridge 100 containing twelve independent sample lanes 101 capable of simultaneous or successive processing. The microfluidic network in each lane is typically configured to carry out amplification, such as by PCR, on a PCR-ready sample, such as one containing nucleic acid extracted from a 65 sample using other methods as further described herein. A PCR-ready sample is thus typically a mixture comprising the

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PCR reagents and the neutralized polynucleotide sample, suitable for subjecting to thermal cycling conditions that create PCR amplicons from the neutralized polynucleotide sample. For example, a PCR-ready sample can include a PCR reagent mixture comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid and a plurality of nucleotides, and at least one probe that is selective for a polynucleotide sequence. Exemplary probes are further described herein. Typically, the microfluidic network is configured to couple heat from an external heat source with the mixture comprising the PCR reagent and the neutralized polynucleotide sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide sample.

In various embodiments, the PCR reagent mixture can include a positive control plasmid and a plasmid fluorogenic hybridization probe selective for at least a portion of the plasmid, and the microfluidic cartridge can be configured to allow independent optical detection of the fluorogenic hybridization probe and the plasmid fluorogenic hybridization probe.

In various embodiments, the microfluidic cartridge can <sup>25</sup> accommodate a negative control polynucleotide, wherein the microfluidic network can be configured to independently carry out PCR on each of a neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide. Each lane of a multi-lane cartridge as described herein can perform two reactions when used in conjunction with two fluorescence detection systems per lane. A variety of combinations of reactions can be performed in the cartridge, such as two sample reactions in one lane, a positive control and a negative control in two other lanes; or a sample reaction and an internal control in one lane and a negative control in a separate lane.

FIG. 2A shows a perspective view of a portion of an exemplary microfluidic cartridge 200 according to the present technology. FIG. 2B shows a close-up view of a portion of the cartridge 200 of FIG. 2A illustrating various representative components. The cartridge 200 may be referred to as a multilane PCR cartridge with dedicated sample inlets 202. For example sample inlet 202 is configured to accept a liquid transfer member (not shown) such as a syringe, a pipette, or a PCR tube containing a PCR ready sample. More than one inlet 202 is shown in FIGS. 2A, 2B, wherein one inlet operates in conjunction with a single sample lane. Various components of microfluidic circuitry in each lane are also visible. For example, microvalves 204, and 206, and hydrophobic vents 208 for removing air bubbles, are parts of microfluidic circuitry in a given lane. Also shown is an ultrafast PCR reactor 210, which, as further described herein, is a microfluidic channel in a given sample lane that is long enough to permit PCR to amplify polynucleotides present in a sample. Above each PCR reactor 210 is a window 212 that permits detection of fluorescence from a fluorescent substance in PCR reactor 210 when a detector is situated above window 212. It is to be understood that other configurations of windows are possible including, but not limited to, a single window that straddles each PCR reactor across the width of cartridge 200.

In preferred embodiments, the multi-sample cartridge has a size substantially the same as that of a 96-well plate as is

customarily used in the art. Advantageously, then, such a cartridge may be used with plate handlers used elsewhere in

The sample inlets of adjacent lanes are reasonably spaced apart from one another to prevent any contamination of one 5 sample inlet from another sample when a user introduces a sample into any one cartridge. In an embodiment, the sample inlets are configured so as to prevent subsequent inadvertent introduction of sample into a given lane after a sample has already been introduced into that lane. In certain embodiments, the multi-sample cartridge is designed so that a spacing between the centroids of sample inlets is 9 mm, which is an industry-recognized standard. This means that, in certain embodiments the center-to-center distance between inlet holes in the cartridge that accept samples from PCR tubes, as 15 further described herein, is 9 mm. The inlet holes can be manufactured conical in shape with an appropriate conical angle so that industry-standard pipette tips  $(2 \mu l, 20 \mu l, 200 \mu l,$ volumes, etc.) fit snugly therein. The cartridge herein may be adapted to suit other, later-arising, industry standards not 20 layer 428, 430 of polypropylene or other plastic label with otherwise described herein, as would be understood by one of ordinary skill in the art.

FIG. 3 shows a plan view of an exemplary microfluidic cartridge 300 having 12 sample lanes. The inlet ports 302 in this embodiment have a 6 mm spacing, so that, when used in 25 conjunction with an automated sample loader having 4 heads, spaced equidistantly at 18 mm apart, the inlets can be loaded in three batches of four inlets: e.g., inlets 1, 4, 7, and 10 together, followed by 2, 5, 8, and 11, then finally 3, 6, 9, and 12, wherein the 12 inlets are numbered consecutively from 30 one side of the cartridge to the other as shown.

A microfluidic cartridge as used herein may be constructed from a number of layers. Accordingly, one aspect of the present technology relates to a microfluidic cartridge that comprises a first, second, third, fourth, and fifth layers 35 wherein one or more layers define a plurality of microfluidic networks, each network having various components configured to carry out PCR on a sample in which the presence or absence of one or more polynucleotides is to be determined. In various embodiments, one or more such layers are 40 optional.

FIGS. 4A-C show various views of a layer structure of an exemplary microfluidic cartridge comprising a number of layers, as further described herein. FIG. 4A shows an exploded view; FIG. 4B shows a perspective view; and FIG. 45 4C shows a cross-sectional view of a sample lane in the exemplary cartridge. Referring to FIGS. 4A-C, an exemplary microfluidic cartridge 400 includes first 420, second 422, third 424, fourth 426, and fifth layers in two non-contiguous parts 428, 430 (as shown) that enclose a microfluidic network 50 having various components configured to process multiple samples in parallel that include one or more polynucleotides to be determined.

Microfluidic cartridge 400 can be fabricated as desired. The cartridge can include a microfluidic substrate layer 424, 55 typically injection molded out of a plastic, such as a zeonor plastic (cyclic olefin polymer), having a PCR channel and valve channels on a first side and vent channels and various inlet holes, including wax loading holes and liquid inlet holes, on a second side (disposed toward hydrophobic vent mem- 60 brane 426). It is advantageous that all the microfluidic network defining structures, such as PCR reactors, valves, inlet holes, and air vents, are defined on the same single substrate **424**. This attribute facilitates manufacture and assembly of the cartridge. Additionally, the material from which this sub- 65 strate is formed is rigid or non-deformable, non-venting to air and other gases, and has a low autofluorescence to facilitate

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detection of polynucleotides during an amplification reaction performed in the microfluidic circuitry defined therein. Rigidity is advantageous because it facilitates effective and uniform contact with a heat unit as further described herein. Use of a non-venting material is also advantageous because it reduces the likelihood that the concentration of various species in liquid form will change during analysis. Use of a material having low auto-fluorescence is also important so that background fluorescence does not detract from measurement of fluorescence from the analyte of interest.

The cartridge can further include, disposed on top of the substrate 424, an oleophobic/hydrophobic vent membrane layer 426 of a porous material, such as 0.2 to 1.0 micron pore-size membrane of modified polytetrafluorethylene, the membrane being typically between about 25 and about 100 microns thick, and configured to cover the vent channels of microfluidic substrate 424, and attached thereto using, for example, heat bonding.

Typically, the microfluidic cartridge further includes a pressure sensitive adhesive (typically between about 50 and 150 microns thick) configured to seal the wax loading holes of the valves in substrate 424, trap air used for valve actuation, and serve as a location for operator markings. In FIG. 4A, this layer is shown in two separate pieces, 428, 430, though it would be understood by one of ordinary skill in the art that a single piece layer would be appropriate.

In various embodiments, the label is a computer-readable label. For example, the label can include a bar code, a radio frequency tag or one or more computer-readable characters. The label can be formed of a mechanically compliant material. For example, the mechanically compliant material of the label can have a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100. The label can be positioned such that it can be read by a sample identification verifier as further described herein.

The cartridge can further include a heat sealable laminate layer 422 (typically between about 100 and about 125 microns thick) attached to the bottom surface of the microfluidic substrate 424 using, for example, heat bonding. This layer serves to seal the PCR channels and vent channels in substrate 424. The cartridge can further include a thermal interface material layer 420 (typically about 125 microns thick), attached to the bottom of the heat sealable laminate layer using, for example, pressure sensitive adhesive. The layer 420 can be compressible and have a higher thermal conductivity than common plastics, thereby serving to transfer heat across the laminate more efficiently. Typically, however, layer 420 is not present.

The application of pressure to contact the cartridge to the heater of an instrument that receives the cartridge generally assists in achieving better thermal contact between the heater and the heat-receivable parts of the cartridge, and also prevents the bottom laminate structure from expanding, as would happen if the PCR channel was only partially filled with liquid and the air entrapped therein would be thermally expanded during thermocycling.

In use, cartridge 400 is typically thermally associated with an array of heat sources configured to operate the components (e.g., valves, gates, actuators, and processing region 410) of the device. Exemplary such heater arrays are further described herein. Additional embodiments of heater arrays are described in U.S. patent application Ser. No. 11/940,315, entitled "Heater Unit for Microfluidic Diagnostic System" and filed on even date herewith, the specification of which is incorporated herein by reference in its entirety. In some embodiments, the heat sources are controlled by a computer

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processor and actuated according to a desired protocol. Processors configured to operate microfluidic devices are described in, e.g., U.S. application Ser. No. 09/819,105, filed Mar. 28, 2001, which application is incorporated herein by reference.

In various embodiments, during transport and storage, the microfluidic cartridge can be further surrounded by a sealed pouch. The microfluidic cartridge can be sealed in the pouch with an inert gas. The microfluidic cartridge can be disposable for example after one or more of its sample lanes have 10 been used.

Highly Multiplexed Embodiments

Embodiments of the cartridge described herein may be constructed that have high-density microfluidic circuitry on a single cartridge that thereby permit processing of multiple 15 samples in parallel, or in sequence, on a single cartridge. Preferred numbers of such multiple samples include 20, 24, 36, 40, 48, 50, 60, 64, 72, 80, 84, 96, and 100, but it would be understood that still other numbers are consistent with the apparatus and cartridge herein, where deemed convenient and 20 practical

Accordingly, different configurations of lanes, sample inlets, and associated heater networks than those explicitly depicted in the FIGs and examples that can facilitate processing such numbers of samples on a single cartridge are within 25 the scope of the instant disclosure. Similarly, alternative configurations of detectors and heating elements for use in conjunction with such a highly multiplexed cartridge are also within the scope of the description herein.

It is also to be understood that the microfluidic cartridges 30 described herein are not to be limited to rectangular shapes, but can include cartridges having circular, elliptical, triangular, rhombohedral, square, and other shapes. Such shapes may also be adapted to include some irregularity, such as a cut-out, to facilitate placement in a complementary apparatus as further described herein.

In an exemplary embodiment, a highly multiplexed cartridge has 48 sample lanes, and permits independent control of each valve in each lane by suitably configured heater circuitry, with 2 banks of thermocycling protocols per lane, as 40 shown in FIG. 5. In the embodiment in FIG. 5, the heaters (shown superimposed on the lanes) are arranged in three arrays 502, 504, with 506, and 508. The heaters are themselves disposed within one or more substrates. Heater arrays **502**, **508** in two separate glass regions only apply heat to 45 valves in the microfluidic networks in each lane. Because of the low thermal conductivity of glass, the individual valves may be heated separately from one another. This permits samples to be loaded into the cartridge at different times, and passed to the PCR reaction chambers independently of one 50 another. The PCR heaters 504, 506 are mounted on a silicon substrate—and are not readily heated individually, but thereby permit batch processing of PCR samples, where multiple samples from different lanes are amplified by the same set of heating/cooling cycles. It is preferable for the PCR 55 heaters to be arranged in 2 banks (the heater arrays 506 on the left and right 508 are not in electrical communication with one another), thereby permitting a separate degree of sample

FIG. 6 shows a representative 48-sample cartridge 600 60 compatible with the heater arrays of FIG. 5, and having a configuration of inlets 602 different to that depicted o other cartridges herein. The inlet configuration is exemplary and has been designed to maximize efficiency of space usage on the cartridge. The inlet configuration can be compatible with 65 an automatic pipetting machine that has dispensing heads situated at a 9 mm spacing. For example, such a machine

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having 4 heads can load 4 inlets at once, in 12 discrete steps, for the cartridge of FIG. 6. Other configurations of inlets though not explicitly described or depicted are compatible with the technology described herein.

FIG. 7 shows, in close up, an exemplary spacing of valves 702, channels 704, and vents 706, in adjacent lanes 708 of a multi-sample microfluidic cartridge for example as shown in FIG. 6.

FIGS. 8 and 9 show close-ups of, respectively, heater arrays 804 compatible with, and inlets 902 on, the exemplary cartridge shown in FIG. 7.

FIGS. 10A and 10B show various views of an embodiment of a radially-configured highly-multiplexed cartridge, having a number of inlets 1002, microfluidic lanes 1004, valves 1005, and PCR reaction chambers 1006. FIG. 10C shows an array of heater elements 1008 compatible with the cartridge layout of FIG. 10A.

The various embodiments shown in FIGS. 5-10C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the other specific examples described herein.

During the design and manufacture of highly multiplexed cartridges, photolithographic processing steps such as etching, hole drilling/photo-chemical drilling/sand-blasting/ion-milling processes should be optimized to give well defined holes and microchannel pattern. Proper distances between channels should be identified and maintained to obtain good bonding between the microchannel substrate and the heat conducting substrate layer. In particular, it is desirable that minimal distances are maintained between pairs of adjacent microchannels to promote, reliable bonding of the laminate in between the channels.

The fabrication by injection molding of these complicated microfluidic structures having multiple channels and multiple inlet holes entails proper consideration of dimensional repeatability of these structures over multiple shots from the injection molding master pattern. Proper consideration is also attached to the placement of ejector pins to push out the structure from the mold without causing warp, bend or stretching of it. For example, impression of the ejector pins on the microfluidic substrate should not sink into the substrate thereby preventing planarity of the surface of the cartridge. The accurate placement of various inlet holes (such as sample inlet holes, valve inlet holes and vent holes) relative to adjacent microfluidic channels is also important because the presence of these holes can cause knit-lines to form that might cause unintended leak from a hole to a microchannel. Highly multiplexed microfluidic substrates may be fabricated in other materials such as glass, silicon.

The size of the substrate relative to the number of holes is also factor during fabrication because it is easy to make a substrate having just a simple microfluidic network with a few holes (maybe fewer than 10 holes) and a few microchannels, but making a substrate having over 24, or over 48, or over 72 holes, etc., is more difficult.

Microfluidic Networks

Particular components of exemplary microfluidic networks are further described herein.

Channels of a microfluidic network in a lane of cartridge typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

FIG. 11 shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. 2A and 2B. It would be understood by one

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skilled in the art that other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In operation of the cartridge, in sequence, sample is introduced through liquid inlet 202, optionally flows into a bubble removal vent channel 208 (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel 216. Typically, when using a robotic dispenser of liquid sample, the volume is dispensed accurately enough that formation of bubbles is not a significant problem, and the presence of vent channel 208 is not necessary. Thus, in certain embodiments, the bubble removal vent channel 208 is not present and sample flows directly into channel 216. Throughout the operation of cartridge 200, the fluid is manipulated as a microdroplet (not shown in FIG. 11). Valves 204 and 206 are initially both open, so that a microdroplet of sample-containing fluid can be pumped into PCR reactor channel 210 from inlet hole 202 under influence of force from the sample injection operation. Upon initiating of processing, the detector 20 present on top of the PCR reactor 210 checks for the presence of liquid in the PCR channel, and then valves 204 and 206 are closed to isolate the PCR reaction mix from the outside. In one embodiment, the checking of the presence of liquid in the PCR channel is by measuring the heat ramp rate, such as by 25 one or more temperature sensors in the heating unit. A channel with liquid absent will heat up faster than one in which, e.g., a sample, is present.

Both valves 204 and 206 are closed prior to thermocycling to prevent or reduce any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor. End vent 214 is configured to prevent a user from introducing an excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample 35 volumes as small as an amount to fill the region from the bubble removal vent (if present) to the middle of the microreactor, or up to valve 204 or beyond valve 204. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction.

The reactor 210 is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein, and according to amplification protocols known to those of ordinary skill 45 in the art. The inside walls of the channel in the PCR reactor are typically made very smooth and polished to a shiny finish (for example, using a polish selected from SPI A1, SPI A2, SPI A3, SPI B1, or SPI B2) during manufacture. This is in order to minimize any microscopic quantities of air trapped in 50 the surface of the PCR channel, which would causing bubbling during the thermocycling steps. The presence of bubbles especially in the detection region of the PCR channel could also cause a false or inaccurate reading while monitoring progress of the PCR. Additionally, the PCR channel can 55 be made shallow such that the temperature gradient across the depth of the channel is minimized.

The region of the cartridge 212 above PCR reactor 210 is a thinned down section to reduce thermal mass and autofluorescence from plastic in the cartridge. It permits a detector to 60 more reliably monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. Exemplary probes are further described herein. The region 212 can be made of thinner material than the rest of the cartridge so as to permit the PCR channel to be 65 more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denatur-

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ing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence.

After PCR has been carried out on a sample, and presence or absence of a polynucleotide of interest has been determined, it is preferred that the amplified sample remains in the cartridge and that the cartridge is either used again (if one or more lanes remain unused), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the laminate of the cartridge, and recover an amount—typically about 1.5 microliter—of PCR product. The user may also place the individual PCR lane on a special narrow heated plate, maintained at a temperature to melt the wax in the valve, and then aspirate the reacted sample from the inlet hole of that PCR lane

In various embodiments, the microfluidic network can optionally include at least one reservoir configured to contain waste.

Table 1 outlines typical volumes, pumping pressures, and operation times associated with various components of a microfluidic cartridge described herein.

TABLE 1

5	Operation	Pumping n Pressure	Displacement Volume	Time of Operation
	Moving valve wa plugs	~1-2 psi x	<1 µl	5-15 seconds
)	Operation	Pump Used	Pump Design	Pump Actuation
	Moving valve wax plugs	Thermopneumatic pump	1 μl of trapped air	Heat trapped air to ~70-90 C.

Valves

A valve (sometimes referred to herein as a microvalve) is a component in communication with a channel, such that the valve has a normally open state allowing material to pass along a channel from a position on one side of the valve (e.g., upstream of the valve) to a position on the other side of the valve (e.g., downstream of the valve). Upon actuation of the valve, the valve transitions to a closed state that prevents material from passing along the channel from one side of the valve to the other. For example, in one embodiment, a valve can include a mass of a thermally responsive substance (TRS) that is relatively immobile at a first temperature and more mobile at a second temperature. The first and second temperatures are insufficiently high to damage materials, such as polymer layers of a microfluidic cartridge in which the valve is situated. A mass of TRS can be an essentially solid mass or an agglomeration of smaller particles that cooperate to obstruct the passage when the valve is closed. Examples of TRS's include a eutectic alloy (e.g., a solder), wax (e.g., an olefin), polymers, plastics, and combinations thereof. The TRS can also be a blend of variety of materials, such as an emulsion of thermoelastic polymer blended with air microbubbles (to enable higher thermal expansion, as well as reversible expansion and contraction), polymer blended with expancel material (offering higher thermal expansion), polymer blended with heat conducting microspheres (offering faster heat conduction and hence, faster melting profiles), or a polymer blended with magnetic microspheres (to permit magnetic actuation of the melted thermoresponsive material).

Generally, for such a valve, the second temperature is less than about 90° C. and the first temperature is less than the second temperature (e.g., about 70° C. or less). Typically, a

chamber is in gaseous communication with the mass of TRS. The valve is in communication with a source of heat that can be selectively applied to the chamber of air and to the TRS. Upon heating gas (e.g., air) in the chamber and heating the mass of TRS to the second temperature, gas pressure within the chamber due to expansion of the volume of gas, forces the mass to move into the channel, thereby obstructing material from passing therealong.

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An exemplary valve is shown in FIG. 12A. The valve of FIG. 12A has two chambers of air 1203, 1205 in contact with, 10 respectively, each of two channels 1207, 1208 containing TRS. The air chambers also serve as loading ports for TRS during manufacture of the valve, as further described herein. In order to make the valve sealing very robust and reliable, the flow channel 1201 (along which, e.g., sample passes) at the 15 valve junction is made narrow (typically 150 μm wide, and 150 µm deep or narrower), and the constricted portion of the flow channel is made at least 0.5 or 1 mm long such that the TRS seals up a long narrow channel thereby reducing any leakage through the walls of the channel. In the case of a bad 20 seal, there may be leakage of fluid around walls of channel, past the TRS, when the valve is in the closed state. In order to minimize this, the flow channel is narrowed and elongated as much as possible. In order to accommodate such a length of channel on a cartridge where space may be at a premium, the 25 flow channel can incorporate one or more curves 1209 as shown in FIG. 12A. The valve operates by heating air in the TRS-loading port, which forces the TRS forwards into the flow-channel in a manner so that it does not come back to its original position. In this way, both air and TRS are heated 30 during operation.

In various other embodiments, a valve for use with a microfluidic network in a microfluidic cartridge herein can be a bent valve as shown in FIG. 12B. Such a configuration reduces the footprint of the valve and hence reduces cost per 35 part for highly dense microfluidic cartridges. A single valve loading hole 1211 is positioned in the center, that serves as an inlet for thermally responsive substance. The leftmost vent 1213 can be configured to be an inlet for, e.g., sample, and the rightmost vent 1215 acts as an exit for, e.g., air. This configuration can be used as a prototype for testing such attributes as valve and channel geometry and materials.

In various other embodiments, a valve for use with a microfluidic network can include a curved valve as shown in FIG. 12C, in order to reduce the effective cross-section of the 45 valve, thereby enabling manufacture of cheaper dense microfluidic devices. Such a valve can function with a single valve loading hole and air chamber 1221 instead of a pair as shown in FIG. 12A.

Gates

FIG. 12D shows an exemplary gate as may optionally be used in a microfluidic network herein. A gate can be a component that can have a closed state that does not allow material to pass along a channel from a position on one side of the gate to another side of the gate, and an open state that does allow material to pass along a channel from a position on one side of the gate to another side of the gate. Actuation of an open gate can transition the gate to a closed state in which material is not permitted to pass from one side of the gate (e.g., upstream of the gate). Upon actuation, a closed gate can transition to an open state in which material is permitted to pass from one side of the gate (e.g., upstream of the gate) to the other side of the gate) to the other side of the gate (e.g., downstream of the gate) to the other side of the gate (e.g., downstream of the gate).

In various embodiments, a microfluidic network can 65 description herein. include a narrow gate 380 as shown in FIG. 12D where a gate

A width of the typically at least all typically

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hole 384 to a gate junction 386 can be narrower (e.g., approximately 150 µm wide and 100 microns deep). An upstream channel 388 as well as a downstream channel 390 of the gate junction 386 can be made wide (e.g., ~500 μm) and deep (e.g.,  $\sim$ 500  $\mu$ m) to help ensure the wax stops at the gate junction 386. The amount of gate material melted and moved out of the gate junction 386 may be minimized for optimal gate 380 opening. As an off-cartridge heater may be used to melt the thermally responsive substance in gate 380, a misalignment of the heater could cause the wax in the gate loading channel 382 to be melted as well. Therefore, narrowing the dimension of the loading channel may increase reliability of gate opening. In the case of excessive amounts of wax melted at the gate junction 386 and gate loading channel 382, the increased cross-sectional area of the downstream channel 390 adjacent to the gate junction 386 can prevent wax from clogging the downstream channel 390 during gate 380 opening. The dimensions of the upstream channel 388 at the gate junction 386 can be made similar to the downstream channel 390 to ensure correct wax loading during gate fabrication.

In various embodiments, the gate can be configured to minimize the effective area or footprint of the gate within the network and thus bent gate configurations, although not shown herein are consistent with the foregoing description. Vents

In various embodiments, the microfluidic network can include at least one hydrophobic vent in addition to an end vent. A vent is a general outlet (hole) that may or may not be covered with a hydrophobic membrane. An exit hole is an example of a vent that need not be covered by a membrane.

A hydrophobic vent (e.g., a vent in FIG. 13) is a structure that permits gas to exit a channel while limiting (e.g., preventing) quantities of liquid from exiting the channel. Typically, hydrophobic vents include a layer of porous hydrophobic material (e.g., a porous filter such as a porous hydrophobic membrane from GE Osmonics, Minnetonka, Minn.) that defines a wall of the channel. As described elsewhere herein, hydrophobic vents can be used to position a microdroplet of sample at a desired location within a microfluidic network.

The hydrophobic vents of the present technology are preferably constructed so that the amount of air that escapes through them is maximized while minimizing the volume of the channel below the vent surface. Accordingly, it is preferable that the vent is constructed so as to have a hydrophobic membrane 1303 of large surface area and a shallow cross section of the microchannel below the vent surface.

Hydrophobic vents are useful for bubble removal and typically have a length of at least about 2.5 mm (e.g., at least about 5 mm, at least about 7.5 mm) along a channel **1305** (see FIG. **13**). The length of the hydrophobic vent is typically at least about 5 times (e.g., at least about 10 times, at least about 20 times) larger than a depth of the channel within the hydrophobic vent. For example, in some embodiments, the channel depth within the hydrophobic vent is about 300 microns or less (e.g., about 250 microns or less, about 200 microns or less, about 150 microns or less).

The depth of the channel within the hydrophobic vent is typically about 75% or less (e.g., about 65% or less, about 60% or less) of the depth of the channel upstream **1301** and downstream (not shown) of the hydrophobic vent. For example, in some embodiments the channel depth within the hydrophobic vent is about 150 microns and the channel depth upstream and downstream of the hydrophobic vent is about 250 microns. Other dimensions are consistent with the description herein.

A width of the channel within the hydrophobic vent is typically at least about 25% wider (e.g., at least about 50%

wider) than a width of the channel upstream from the vent and downstream from the vent. For example, in an exemplary embodiment, the width of the channel within the hydrophobic vent is about 400 microns, and the width of the channel upstream and downstream from the vent is about 250 5 microns. Other dimensions are consistent with the description herein.

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The vent in FIG. 13 is shown in a linear configuration though it would be understood that it need not be so. A bent, kinked, curved, S-shaped, V-shaped, or U-shaped (as in item 10 208 FIG. 11) vent is also consistent with the manner of construction and operation described herein.

Heater Configurations to Ensure Uniform Heating of a Region

The microfluidic cartridges described herein are configured to position in a complementary receiving bay in an apparatus that contains a heater unit. The heater unit is configured to deliver heat to specific regions of the cartridge, including but not limited to one or more microfluidic components, at specific times. For example, the heat source is configured so that particular heating elements are situated adjacent to specific components of the microfluidic network of the cartridge. In certain embodiments, the apparatus uniformly controls the heating of a region of a microfluidic network. In an exemplary embodiment, multiple heaters can be configured to simultaneously and uniformly heat a single region, such as the PCR reaction chamber, of the microfluidic cartridge. In other embodiments, portions of different sample lanes are heated simultaneously and independently of one another.

FIG. 14 shows a cross-sectional view of an exemplary microfluidic cartridge to show the location of a PCR channel in relation to various heaters when the cartridge is placed in a suitable apparatus. The view in FIG. 14 is also referred to as a sectional-isometric view of the cartridge lying over a heater 35 wafer. A window 903 above the PCR channel in the cartridge is shown in perspective view. PCR channel 901 (for example, 150μ deep×700μ wide), is shown in an upper layer of the cartridge. A laminate layer 905 of the cartridge (for example, 125μ thick) is directly under the PCR channel 901. As 40 depicted, an optional further layer of thermal interface laminate 907 on the cartridge (for example, 125µ thick) lies directly under the laminate layer 905. Heaters 909, 911 are situated in a heater substrate layer 913 directly under the thermal interface laminate, shown in cross-section. In one 45 embodiment the heaters are photolithographically defined and etched metal layers of gold (typically about 3,000 Å thick). Layers of 400 Å of TiW (not shown) are deposited on top and bottom of the gold layer to serve as an adhesion layer. The substrate 913 used is glass, fused silica or a quartz wafer 50 having a thickness of 0.4 mm, 0.5 mm, 0.7 mm, or 1 mm. A thin electrically-insulative layer of 2 µm silicon oxide serves as an insulative layer on top of the metal layer. Additional thin electrically insulative layers such as 2-4 μm of Parylene may also be deposited on top of the silicon oxide surface. Two long 55 heaters 909 and 911, as further described herein, run alongside the PCR channel.

An exemplary heater array is shown in FIGS. **15**A and **15**B. Additional embodiments of heater arrays are described in U.S. patent application Ser. No. 11/940,315, entitled "Heater 60 Unit for Microfluidic Diagnostic System" and filed on even date herewith, the specification of which is incorporated herein by reference in its entirety.

Referring to FIGS. 15A and 15B, an exemplary PCR reaction chamber 1501, typically having a volume  $\sim$ 1.6  $\mu$ l, is 65 configured with a long side and a short side, each with an associated heating element. The heater substrate therefore

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includes four heaters disposed along the sides of, and configured to heat, the PCR reaction chamber, as shown in the exemplary embodiment of FIG. 15A: long top heater 1505, long bottom heater 1503, short left heater 1507, and short right heater 1509. The small gap between long top heater 1505 and long bottom heater 1503 results in a negligible temperature gradient (less than 1° C. difference across the width of the PCR channel at any point along the length of the PCR reaction chamber) and therefore an effectively uniform temperature throughout the PCR reaction chamber. The heaters on the short edges of the PCR reactor provide heat to counteract the gradient created by the two long heaters from the center of the reactor to the edge of the reactor. It would be understood by one of ordinary skill in the art that still other configurations of one or more heater(s) situated about a PCR reaction chamber are consistent with the methods and apparatus described herein. For example, a 'long' side of the reaction chamber can be configured to be heated by two or more heaters. Specific orientations and configurations of heaters are used to create uniform zones of heating even on substrates having poor thermal conductivity because the poor thermal conductivity of glass, or quartz, polyimide, FR4, ceramic, or fused silica substrates is utilized to help in the independent operation of various microfluidic components such as valves and independent operation of the various PCR lanes. In FIG. 15B, various aspects of fine structure of heater elements are shown in inserts.

Generally, the heating of microfluidic components, such as a PCR reaction chamber, is controlled by passing currents through suitably configured microfabricated heaters. Under control of suitable circuitry, the lanes of a multi-lane cartridge can then be controlled independently of one another. This can lead to a greater energy efficiency of the apparatus, because not all heaters are heating at the same time, and a given heater is receiving current for only that fraction of the time when it is required to heat. Control systems and methods of controllably heating various heating elements are further described in U.S. patent application Ser. No. 11/940,315, filed Nov. 14, 2007 and entitled "Heater Unit for Microfluidic Diagnostic System"

The configuration for uniform heating, shown in FIG. 15A for a single PCR reaction chamber, can be applied to a multilane PCR cartridge in which multiple independent PCR reactions occur. See, e.g., FIG. 15C, which shows an array of heater elements suitable to heat the cartridge of FIG. 1.

In other embodiments, as further described in U.S. patent application Ser. No. 11/940,315, filed Nov. 14, 2007 and entitled "Heater Unit for Microfluidic Diagnostic System", the heaters may have an associated temperature sensor, or may themselves function as sensors.

Use of Cutaways in Cartridge and Substrate to Improve Rate of Cooling During PCR Cycling

During a PCR amplification of a nucleotide sample, a number of thermal cycles are carried out. For improved efficiency, the cooling between each application of heat is preferably as rapid as possible. Improved rate of cooling can be achieved with various modifications to the heating substrate and/or the cartridge, as shown in FIG. 16.

One way to achieve rapid cooling is to cutaway portions of the microfluidic cartridge substrate, as shown in FIG. 16. The upper panel of FIG. 16 is a cross-section of an exemplary microfluidic cartridge taken along the dashed line A-A' as marked on the lower panel of FIG. 16. PCR reaction chamber 1601, and representative heaters 1603 are shown. Also shown are two cutaway portions, one of which labeled 1601, that are situated alongside the heaters that are positioned along the long side of the PCR reaction chamber. Cutaway portions

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such as **1601** reduce the thermal mass of the cartridge, and also permit air to circulate within the cutaway portions. Both of these aspects permit heat to be conducted away quickly from the immediate vicinity of the PCR reaction chamber. Other configurations of cutouts, such as in shape, position, 5 and number, are consistent with the present technology.

Another way to achieve rapid cooling is to cutaway portions of the heater substrate, and also to use ambient air cooling, as further described in U.S. patent application Ser. No. 11/940,315, filed Nov. 14, 2007 and entitled "Heater Unit 10 for Microfluidic Diagnostic System".

An example of thermal cycling performance in a PCR reaction chamber obtained with a configuration as described herein, is shown in FIG. 17 for a protocol that is set to heat up the reaction mixture to 92° C., and maintain the temperature 15 for 1 second, then cool to 62° C., and stay for 10 seconds. The cycle time shown is about 29 seconds, with 8 seconds required to heat from 62° C. and stabilize at 92° C., and 10 seconds required to cool from 92° C., and stabilize at 62° C. To minimize the overall time required for a PCR effective to 20 produce detectable quantities of amplified material, it is important to minimize the time required for each cycle. Cycle times in the range 15-30 s, such as 18-25 s, and 20-22 s, are desirable. In general, an average PCR cycle time of 25 seconds as well as cycle times as low as 20 seconds are typical 25 with the technology described herein. Using reaction volumes less than a microliter (such as a few hundred nanoliters or less) permits use of an associated smaller PCR chamber, and enables cycle times as low as 15 seconds.

Manufacturing Process for Cartridge

FIG. 18 shows a flow-chart 1800 for an embodiment of an assembly process for an exemplary cartridge as shown in FIG. 4A herein. It would be understood by one of ordinary skill in the art, both that various steps may be performed in a different order from the order set forth in FIG. 18, and additionally that 35 any given step may be carried out by alternative methods to those described in the figure. It would also be understood that, where separate serial steps are illustrated for carrying out two or more functions, such functions may be performed synchronously and combined into single steps and remain consistent 40 with the overall process described herein.

At **1802**, a laminate layer is applied to a microfluidic substrate that has previously been engineered, for example by injection molding, to have a microfluidic network constructed in it; edges are trimmed from the laminate where they spill 45 over the bounds of the substrate.

At 1804, wax is dispensed and loaded into the microvalves of the microfluidic network in the microfluidic substrate. An exemplary process for carrying this out is further described herein.

At **1806**, the substrate is inspected to ensure that wax from step **1804** is loaded properly and that the laminate from step **1802** adheres properly to it. If a substrate does not satisfy either or both of these tests, it is usually discarded. If substrates repeatedly fail either or both of these tests, then the 55 wax dispensing, or laminate application steps, as applicable, are reviewed.

At **1808**, a hydrophobic vent membrane is applied to, and heat bonded to, the top of the microfluidic substrate covering at least the one or more vent holes, and on the opposite face of 60 the substrate from the laminate. Edges of the membrane that are in excess of the boundary of the substrate are trimmed.

At **1810**, the assembly is inspected to ensure that the hydrophobic vent membrane is bonded well to the microfluidic substrate without heat-clogging the microfluidic channels. If 65 any of the channels is blocked, or if the bond between the membrane and the substrate is imperfect, the assembly is

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discarded, and, in the case of repeated discard events, the foregoing process step 1808 is reviewed.

At 1812, optionally, a thermally conductive pad layer is applied to the bottom laminate of the cartridge.

At **1814**, two label strips are applied to the top of the microfluidic substrate, one to cover the valves, and a second to protect the vent membranes. It would be understood that a single label strip may be devised to fulfill both of these roles.

At 1816, additional labels are printed or applied to show identifying characteristics, such as a barcode #, lot # and expiry date on the cartridge. Preferably one or more of these labels has a space and a writable surface that permits a user to make an identifying annotation on the label, by hand.

Optionally, at 1818, to facilitate transport and delivery to a customer, assembled and labeled cartridges are stacked, and cartridges packed into groups, such as groups of 25, or groups of 10, or groups of 20, or groups of 48 or 50. Preferably the packaging is via an inert and/or moisture-free medium. Wax Loading in Valves

In general, a valve as shown in, e.g., FIGS. 12A-C, is constructed by depositing a precisely controlled amount of a TRS (such as wax) into a loading inlet machined in the microfluidic substrate. FIGS. 19A and 19B show how a combination of controlled hot drop dispensing into a heated microchannel device of the right dimensions and geometry is used to accurately load wax into a microchannel of a microfluidic cartridge to form a valve. The top of FIG. 19A shows a plan view of a valve inlet 1901 and loading channel 1902, connecting to a flow channel 1904. The lower portions of FIG. 19A show the progression of a dispensed wax droplet 1906 (having a volume of 75 nl±15 nl) through the inlet 1901 and into the loading channel 1902.

To accomplish those steps, a heated dispenser head can be accurately positioned over the inlet hole of the microchannel in the microfluidic device, and can dispense molten wax drops in volumes as small as 75 nanoliters with an accuracy of 20%. A suitable dispenser is also one that can deposit amounts smaller than 100 nl with a precision of +/-20%. The dispenser should also be capable of heating and maintaining the dispensing temperature of the TRS to be dispensed. For example, it may have a reservoir to hold the solution of TRS. It is also desirable that the dispense head can have freedom of movement at least in a horizontal (x-y) plane so that it can easily move to various locations of a microfluidic substrate and dispense volumes of TRS into valve inlets at such locations without having to be re-set, repositioned manually, or recalibrated in between each dispense operation.

The inlet hole of the microfluidic cartridge, or other microchannel device, is dimensioned in such a way that the droplet of 75 nl can be accurately propelled to the bottom of the inlet hole using, for example, compressed air, or in a manner similar to an inkjet printing method. The microfluidic cartridge is maintained at a temperature above the melting point of the wax thereby permitting the wax to stay in a molten state immediately after it is dispensed. After the drop falls to the bottom of the inlet hole 1901, the molten wax is drawn into the narrow channel by capillary action, as shown in the sequence of views in FIG. 19B. A shoulder between the inlet hole 1901 and the loading channel can facilitate motion of the TRS. The volume of the narrow section can be designed to be approximately equal to a maximum typical amount that is dispensed into the inlet hole. The narrow section can also be designed so that even though the wax dispensed may vary considerably between a minimum and a maximum shot size, the wax always fills up to, and stops at, the microchannel junction 1907 because the T-junction provides a higher cross

section than that of the narrow section and thus reduces the capillary forces. Dimensions shown in FIG. 19A are exemplary.

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PCR Reagent Mixtures

In various embodiments, the sample for introduction into a 5 lane of the microfluidic cartridge can include a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides.

In various embodiments, preparation of a PCR-ready sample for use with an apparatus and cartridge as described herein can include contacting a neutralized polynucleotide sample with a PCR reagent mixture comprising a polymerase enzyme and a plurality of nucleotides (in some embodiments, the PCR reagent mixture can further include a positive control least a portion of the plasmid).

The PCR-ready sample can be prepared, for example, using the following steps: (1) collect sample in sample collection buffer, (2) transfer entire sample to lysis tube, mix, heat, and incubate for seven minutes, (3) place on magnetic 20 rack, allow beads to separate, aspirate supernatant, (4) add 100 μl of Buffer 1, mix, place on magnetic rack, allow beads to separate, aspirate supernatant, (5) add 10 μl of Buffer 2, mix, place in high temperature heat block for 3 minutes, place on magnetic rack, allow beads to separate, transfer 5 µl super- 25 natant, and (6) Add 5 μl of Buffer 3, transfer 1 to 10 μl of supernatant for PCR amplification and detection.

The PCR reagent mixture can be in the form of one or more lyophilized pellets and the steps by which the PCR-ready sample is prepared can involve reconstituting the PCR pellet 30 by contacting it with liquid to create a PCR reagent mixture solution. In yet another embodiment, each of the PCR lanes may have dried down or lyophilized ASR reagents preloaded such that the user only needs to input prepared polynucleotide sample into the PCR. In another embodiment, the PCR lanes 35 may have only the application-specific probes and primers pre-measured and pre-loaded, and the user inputs a sample mixed with the PCR reagents.

In various embodiments, the PCR-ready sample can include at least one probe that can be selective for a poly- 40 nucleotide sequence, wherein the steps by which the PCRready sample is prepared involve contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the probe. The probe can be a fluorogenic hybridization probe. The fluorogenic hybridization probe can include a polynucle-45 otide sequence coupled to a fluorescent reporter dye and a fluorescence quencher dve.

In various embodiments, the PCR-ready sample further includes a sample buffer.

In various embodiments, the PCR-ready sample includes at 50 least one probe that is selective for a polynucleotide sequence, e.g., the polynucleotide sequence that is characteristic of a pathogen selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and

In various embodiments, the PCR reagent mixture can further include a polymerase enzyme, a positive control plasmid and a fluorogenic hybridization probe selective for at least a portion of the plasmid.

In various embodiments, the probe can be selective for a 60 polynucleotide sequence that is characteristic of an organism, for example any organism that employs deoxyribonucleic acid or ribonucleic acid polynucleotides. Thus, the probe can be selective for any organism. Suitable organisms include mammals (including humans), birds, reptiles, amphibians, 65 fish, domesticated animals, wild animals, extinct organisms, bacteria, fungi, viruses, plants, and the like. The probe can

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also be selective for components of organisms that employ their own polynucleotides, for example mitochondria. In some embodiments, the probe is selective for microorganisms, for example, organisms used in food production (for example, yeasts employed in fermented products, molds or bacteria employed in cheeses, and the like) or pathogens (e.g., of humans, domesticated or wild mammals, domesticated or wild birds, and the like). In some embodiments, the probe is selective for organisms selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of Staphylococcus spp., plasmid and a fluorogenic hybridization probe selective for at 15 e.g., S. epidermidis, S. aureus, Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant Staphylococcus; Streptococcus (e.g.,  $\alpha$ ,  $\beta$  or  $\gamma$ -hemolytic, Group A, B, C, D or G) such as S. pyogenes, S. agalactiae; E. faecalis, E. durans, and E. faecium (formerly S. faecalis, S. durans, S. faecium); nonenterococcal group D streptococci, e.g., S. bovis and S. equines; Streptococci viridans, e.g., S. mutans, S. sanguis, S. salivarius, S. mitior, A. milleri, S. constellatus, S. intermedius, and S. anginosus; S. iniae; S. pneumoniae; Neisseria, e.g., N. meningitides, N. gonorrhoeae, saprophytic Neisseria sp; Erysipelothrix, e.g., E. rhusiopathiae; Listeria spp., e.g., L. monocytogenes, rarely L. ivanovii and L. seeligeri; Bacillus, e.g., B. anthracis, B. cereus, B. subtilis, B. subtilus niger, B. thuringiensis; Nocardia asteroids; Legionella, e.g., L. pneumonophilia, Pneumocystis, e.g., P. carinii; Enterobacteriaceae such as Salmonella, Shigella, Escherichia (e.g., E. coli, E. coliO157:H7); Klebsiella, Enterobacter, Serratia, Proteus, Morganella, Providencia, Yersinia, and the like, e.g., Salmonella, e.g., S. typhi S. paratyphi A, B (S. schottmuelleri), and C (S. hirschfeldii), S. dublin S. choleraesuis, S. enteritidis, S. typhimurium, S. heidelberg, S. newport, S. infantis, S. agona, S. montevideo, and S. saint-paul; Shigella e.g., subgroups: A, B, C, and D, such as S. flexneri, S. sonnei, S. boydii, S. dysenteriae; Proteus (P. mirabilis, P. vulgaris, and P. myxofaciens), Morganella (M. morganii); Providencia (P. rettgeri, P. alcalifaciens, and P. stuartii); Yersinia, e.g., Y. pestis, Y. enterocolitica; Haemophilus, e.g., H. influenzae, H. parainfluenzae H. aphrophilus, H. ducreyi; Brucella, e.g., B. abortus, B. melitensis, B. suis, B. canis; Francisella, e.g., F. tularensis; Pseudomonas, e.g., P. aeruginosa, P. paucimobilis, P. putida, P. fluorescens, P. acidovorans, Burkholderia (Pseudomonas) pseudomallei, Burkholderia Burkholderia cepacia and Stenotrophomonas maltophilia; Campylobacter, e.g., C. fetus fetus, C. jejuni, C. pylori (Helicobacter pylori); Vibrio, e.g., V. cholerae, V. parahaemolyticus, V. mimicus, V. alginolyticus, V. hollisae, V. vulnificus, and the nonagglutinable vibrios; Clostridia, e.g., C. perfringens, C. tetani, C. difficile, C. botulinum; Actinomyces, e.g., A. israelii; Bacteroides, e.g., B. fragilis, B. thetaiotaomicron, B. 55 distasonis, B. vulgatus, B. ovatus, B. caccae, and B. merdae; Prevotella, e.g., P. melaminogenica; genus Fusobacterium; Treponema, e.g. T. pallidum subspecies endemicum, T. pallidum subspecies pertenue, T. carateum, and T. pallidum subspecies pallidum; genus Borrelia, e.g., B burgdorferi; genus Leptospira; Streptobacillus, e.g., S. moniliformis; Spirillum, e.g., S. minus; Mycobacterium, e.g., M. tuberculosis, M. bovis, M. africanum, M. avium M. intracellulare, M. kansasii, M. xenopi, M. marinum, M. ulcerans, the M. fortuitum complex (M. fortuitum and M. chelonei), M. leprae, M. asiaticum, M. chelonei subsp. abscessus, M. fallax, M. fortuitum, M. malmoense, M. shimoidei, M. simiae, M. szulgai, M. xenopi; Mycoplasma, e.g., M. hominis, M. orale, M. salivarium, M.

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fermentans, M. pneumoniae, M. bovis, M. tuberculosis, M. avium, M. leprae; Mycoplasma, e.g., M. genitalium; Ureaplasma, e.g., U. urealyticum; Trichomonas, e.g., T. vaginalis; Cryptococcus, e.g., C. neoformans; Histoplasma, e.g., H. capsulatum; Candida, e.g., C. albicans; Aspergillus sp; Coc- 5 cidioides, e.g., C. immitis; Blastomyces, e.g. B. dermatitidis; Paracoccidioides, e.g., P. brasiliensis; Penicillium, e.g., P. marneffei; Sporothrix, e.g., S. schenckii; Rhizopus, Rhizomucor, Absidia, and Basidiobolus; diseases caused by Bipolaris, Cladophialophora, Cladosporium, Drechslera, Exophiala, 10 Fonsecaea, Phialophora, Xylohypha, Ochroconis, Rhinocladiella, Scolecobasidium, and Wangiella; Trichosporon, e.g., T. beigelii; Blastoschizomyces, e.g., B. capitatus; Plasmodium, e.g., P. falciparum, P. vivax, P. ovale, and P. malariae; Babesia sp; protozoa of the genus Trypanosoma, e.g., T. 15 cruzi; Leishmania, e.g., L. donovani, L. major L. tropica, L. mexicana, L. braziliensis, L. viannia braziliensis; Toxoplasma, e.g., T. gondii; Amoebas of the genera Naegleria or Acanthamoeba; Entamoeba histolytica; Giardia lamblia; genus Cryptosporidium, e.g., C. parvum; Isospora belli; 20 Cyclospora cayetanensis; Ascaris lumbricoides; Trichuris trichiura; Ancylostoma duodenale or Necator americanus; Strongyloides stercoralis Toxocara, e.g., T. canis, T. cati; Baylisascaris, e.g., B. procyonis; Trichinella, e.g., T. spiralis; Dracunculus, e.g., D. medinensis; genus Filarioidea; 25 Wuchereria bancrofti; Brugia, e.g., B. malayi, or B. timori; Onchocerca volvulus; Loa loa; Dirofilaria immitis; genus Schistosoma, e.g., S. japonicum, S. mansoni, S. mekongi, S. intercalatum, S. haematobium; Paragonimus, e.g., P. Westermani, P. Skriabini; Clonorchis sinensis; Fasciola hepatica; 30 Opisthorchis sp; Fasciolopsis buski; Diphyllobothrium latum; Taenia, e.g., T. saginata, T. solium; Echinococcus, e.g., E. granulosus, E. multilocularis; Picornaviruses, rhinoviruses echoviruses, coxsackieviruses, influenza virus; paramyxoviruses, e.g., types 1, 2, 3, and 4; adnoviruses; Her- 35 pesviruses, e.g., HSV-1 and HSV-2; varicella-zoster virus; human T-lymphotrophic virus (type I and type II); Arboviruses and Arenaviruses; Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae; Flavivirus; Hantavirus; Viral encephalitis (alphaviruses [e.g., Venezuelan equine encephalitis, 40 eastern equine encephalitis, western equine encephalitis]); Viral hemorrhagic fevers (filoviruses [e.g., Ebola, Marburg] and arenaviruses [e.g., Lassa, Machupo]); Smallpox (variola); retroviruses e.g., human immunodeficiency viruses 1 and 2; human papillomavirus [HPV] types 6, 11, 16, 18, 31, 45 33, and 35.

In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of an organisms selected from the group consisting of Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytoca, Klebsiella 50 pneumoniae, Escherichia coli, Acinetobacter Baumannii, Serratia marcescens, Enterobacter aerogenes, Enterococcus faecium, vancomycin-resistant enterococcus (VRE), Staphylococcus aureus, methecillin-resistant Staphylococcus aureus(MRSA), Streptococcus viridans, Listeria monocyto- 55 genes, Enterococcus spp., Streptococcus Group B, Streptococcus Group C, Streptococcus Group G, Streptococcus Group F, Enterococcus faecalis, Streptococcus pneumoniae, Staphylococcus epidermidis, Gardenerella vaginalis, Micrococcus sps., Haemophilus influenzae, Neisseria gonor- 60 rhoeee, Moraxella catarrahlis, Salmonella sps., Chlamydia trachomatis, Peptostreptococcus productus, Peptostreptococcus anaerobius, Lactobacillus fermentum, Eubacterium lentum, Candida glabrata, Candida albicans, Chlamydia spp., Camplobacter spp., Salmonella spp., smallpox (variola 65 major), Yersina Pestis, Herpes Simplex Virus I (HSV I), and Herpes Simplex Virus II (HSV II).

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In various embodiments, the probe can be selective for a polynucleotide sequence that is characteristic of Group B *Strentococcus*.

In various embodiments, a method of carrying out PCR on a sample can further include one of more of the following steps: heating the biological sample in the microfluidic cartridge; pressurizing the biological sample in the microfluidic cartridge at a pressure differential compared to ambient pressure of between about 20 kilopascals and 200 kilopascals, or in some embodiments, between about 70 kilopascals and 110 kilopascals.

In some embodiments, the method for sampling a polynucleotide can include the steps of: placing a microfluidic cartridge containing a PCR-ready sample in a receiving bay of a suitably configured apparatus; carrying out PCR on the sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide in the sample, the PCR-ready sample comprising a polymerase enzyme, a positive control plasmid, a fluorogenic hybridization probe selective for at least a portion of the plasmid, and a plurality of nucleotides; contacting the neutralized polynucleotide sample or a PCR amplicon thereof with the at least one fluorogenic probe that is selective for a polynucleotide sequence, wherein the probe is selective for a polynucleotide sequence that is characteristic of an organism selected from the group consisting of gram positive bacteria, gram negative bacteria, yeast, fungi, protozoa, and viruses; and detecting the fluorogenic probe, the presence of the organism for which the one fluorogenic probe is selective is determined.

Carrying out PCR on a PCR-ready sample can additionally include: independently contacting each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucleotide sample and PCR amplicons of the negative control polynucleotide; and/or contacting the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence.

In various embodiments, a method of using the apparatus and cartridge described herein can further include one or more of the following steps: determining the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corresponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; determining that the sample was contaminated if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof; and/or in some embodiments, wherein the PCR reagent mixture further comprises a positive control plasmid and a plasmid probe selective for at least a portion of the plasmid, the method further including determining that a PCR amplification has occurred if the plasmid probe is detected.

Kit

In various embodiments, the microfluidic cartridge as described herein can be provided in the form of a kit, wherein the kit can include a microfluidic cartridge, and a liquid transfer member (such as a syringe or a pipette). In various embodiments, the kit can further include instructions to employ the liquid transfer member to transfer a sample containing extracted nucleic acid from a sample container via a sample inlet to the microfluidic network on the microfluidic cartridge. In some embodiments, the microfluidic cartridge and the liquid transfer member can be sealed in a pouch with an inert gas.

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Typically when transferring a sample from liquid dispenser, such as a pipette tip, to an inlet on the microfluidic cartridge, a volume of air is simultaneously introduced into the microfluidic network, the volume of air being between about 0.5 mL and about 5 mL. Presence of air in the microfluidic network, however, is not essential to operation of the cartridge described herein.

In various embodiments, the kit can further include at least one computer-readable label on the cartridge. The label can include, for example, a bar code, a radio frequency tag or one 10 or more computer-readable characters. When used in conjunction with a similar computer-readable label on a sample container, such as a vial or a pouch, matching of diagnostic results with sample is thereby facilitated.

In some embodiments, a sample identifier of the apparatus 15 described elsewhere herein is employed to read a label on the microfluidic cartridge and/or a label on the biological sample. Overview of an Apparatus for Receiving a Microfluidic Cartridge

The present technology relates to a cartridge, complemen- 20 tary apparatus, and related methods for amplifying, and carrying out diagnostic analyses on, nucleotides from biological samples. The technology includes a disposable or reusable microfluidic cartridge containing multiple sample lanes capable of processing samples in parallel as described else- 25 where herein, and a reusable apparatus that is configured to selectively actuate on-cartridge operations, to detect and analyze the products of the PCR amplification in each of the lanes separately, in all simultaneously, or in groups simultaneously, and, optionally, can display the progression of analyses and 30 results thereof on a graphical user interface. Such a reusable apparatus is further described in U.S. patent application Ser. No. 11/985,577, entitled "Microfluidic System for Amplifying and Detecting Polynucleotides in Parallel" and filed on Nov. 14, 2007, and which is incorporated herein by reference 35

FIG. 20 shows a perspective view of an exemplary apparatus 2000 consistent with those described herein, as well as various components thereof, such as exemplary cartridge 2010 that contains multiple sample lanes, and exemplary read 40 head 2020 that contains detection apparatus for reading signals from cartridge 2010. The apparatus 2000 of FIG. 20 is able to carry out real-time PCR on a number of samples in cartridge 2010 simultaneously or serially. Preferably the number of samples is 12 samples, as illustrated with exem- 45 plary cartridge 2010, though other numbers of samples such as 4, 8, 10, 16, 20, 24, 25, 30, 32, 36, 40, and 48 are within the scope of the present description. In preferred operation of the apparatus, a PCR-ready solution containing the sample, and, optionally, one or more analyte-specific reagents (ASR's) is 50 prepared, as further described elsewhere (see, e.g., U.S. patent application publication 2006-0166233, incorporated herein by reference), prior to introduction into cartridge 200.

In some embodiments, an apparatus includes: a receiving bay configured to selectively receive a microfluidic cartridge 55 as described herein; at least one heat source thermally coupled to the receiving bay; and a processor coupled to the heat source, wherein the heat source is configured to selectively heat individual regions of individual sample lanes in the cartridge, and the processor is configured to control application of heat to the individual sample lanes, separately, in all simultaneously, or in groups simultaneously; at least one detector configured to detect one or more polynucleotides or a probe thereof in a sample in one or more of the individual sample lanes, separately or simultaneously; and a processor 65 coupled to the detector to control the detector and to receive signals from the detector.

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FIG. 21 shows a schematic cross-sectional view of a part of an apparatus as described herein, showing input of sample into a cartridge 2100 via a pipette 10 (such as a disposable pipette) and an inlet 202. Cartridge 2100 is situated in a suitably configured receiving bay 2112. Inlet 2102 is preferably configured to receive a pipette or the bottom end of a PCR tube and thereby accept sample for analysis with minimum waste, and with minimum introduction of air. Cartridge 2100 is disposed on top of and in contact with a heater substrate 2140. Read head 2130 is positioned above cartridge 2100 and a cover for optics 2131 restricts the amount of ambient light that can be detected by the read head.

FIG. 22 shows an example of 4-pipette head used for attaching disposable pipette tips, prior to dispensing PCR-ready sample into a cartridge as further described herein.

The receiving bay is a portion of the apparatus that is configured to selectively receive the microfluidic cartridge. For example, the receiving bay and the microfluidic cartridge can be complementary in shape so that the microfluidic cartridge is selectively received in, e.g., a single orientation. The microfluidic cartridge can have a registration member that fits into a complementary feature of the receiving bay. The registration member can be, for example, a cut-out on an edge of the cartridge, such as a corner that is cut-off, or one or more notches or grooves that are made on one or more of the sides in a distinctive pattern that prevents a cartridge from being loaded into the bay in more than one distinct orientation. By selectively receiving the cartridge, the receiving bay can help a user to place the cartridge so that the apparatus can properly operate on the cartridge. The cartridge can be designed to be slightly smaller than the dimensions of the receiving bay, for example by approximately 200-300 microns, for easy placement and removal of the cartridge.

The receiving bay can also be configured so that various components of the apparatus that operate on the microfluidic cartridge (heat sources, detectors, force members, and the like) are positioned to properly operate thereon. For example, a contact heat source can be positioned in the receiving bay such that it can be thermally coupled to one or more distinct locations on a microfluidic cartridge that is selectively received in the bay. Microheaters in the heater module as further described elsewhere herein were aligned with corresponding heat-requiring microcomponents (such as valves, pumps, gates, reaction chambers, etc). The microheaters can be designed to be slightly bigger than the heat requiring microfluidic components so that even though the cartridge may be off-centered from the heater, the individual components can still function effectively.

As further described elsewhere herein, the lower surface of the cartridge can have a layer of mechanically compliant heat transfer laminate that can enable thermal contact between the microfluidic substrate and the microheater substrate of the heater module. A minimal pressure of 1 psi can be employed for reliable operation of the thermal valves, gates and pumps present in the microfluidic cartridge.

In various embodiments of the apparatus, the apparatus can further include a sensor coupled to the processor, the sensor configured to sense whether the microfluidic cartridge is selectively received.

The heat source can be, for example, a heat source such as a resistive heater or network of resistive heaters. In preferred embodiments, the at least one heat source can be a contact heat source selected from a resistive heater (or network thereof), a radiator, a fluidic heat exchanger and a Peltier device. The contact heat source can be configured at the receiving bay to be thermally coupled to one or more distinct locations of a microfluidic cartridge received in the receiving

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bay, whereby the distinct locations are selectively heated. The contact heat source typically includes a plurality of contact heat sources, each configured at the receiving bay to be independently thermally coupled to a different distinct location in a microfluidic cartridge received therein, whereby the distinct 5 locations are independently heated. The contact heat sources can be configured to be in direct physical contact with one or more distinct locations of a microfluidic cartridge received in the bay. In various embodiments, each contact source heater can be configured to heat a distinct location having an average diameter in 2 dimensions from about 1 millimeter (mm) to about 15 mm (typically about 1 mm to about 10 mm), or a distinct location having a surface area of between about 1 mm<sup>2</sup> about 225 mm (typically between about 1 mm<sup>2</sup> and about 100 mm<sup>2</sup>, or in some embodiments between about 5 15 mm and about 50 mm<sup>2</sup>). Various configurations of heat sources are further described in U.S. patent application Ser. No. 11/940,315, entitled "Heater Unit for Microfluidic Diagnostic System" and filed on even date herewith.

In various embodiments, the heat source is disposed in a 20 heating module that is configured to be removable from the apparatus.

In various embodiments, the apparatus can include a compliant layer at the contact heat source configured to thermally couple the contact heat source with at least a portion of a 25 microfluidic cartridge received in the receiving bay. The compliant layer can have a thickness of between about 0.05 and about 2 millimeters and a Shore hardness of between about 25 and about 100. Such a compliant layer may not be required if the instrument is able to reliably press the cartridge over the 30 heater surface with a minimum contact pressure of say 1 psi over the entirety of the cartridge.

The detector can be, for example, an optical detector. For example, the detector can include a light source that selectively emits light in an absorption band of a fluorescent dye, 35 and a light detector that selectively detects light in an emission band of the fluorescent dye, wherein the fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof Alternatively, for example, the optical detector can include a bandpass-filtered diode that selectively emits 40 light in the absorption band of the fluorescent dye and a bandpass filtered photodiode that selectively detects light in the emission band of the fluorescent dye; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes having different fluorescent 45 emission spectra, wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof; or for example, the optical detector can be configured to independently detect a plurality of fluorescent dyes at a plurality of different locations on a microfluidic cartridge, 50 wherein each fluorescent dye corresponds to a fluorescent polynucleotide probe or a fragment thereof in a different sample. The detector can also be configured to detect the presence or absence of sample in a PCR reaction chamber in a given sample lane, and to condition initiation of thermocy- 55 cling upon affirmative detection of presence of the sample. Further description of suitably configured detectors are described in U.S. patent application Ser. No. 11/940,321, filed on Nov. 14, 2007 and entitled "Fluorescence Detector for Microfluidic Diagnostic System", incorporated herein by 60

Although the various depictions therein show a heater substrate disposed underneath a microfluidic substrate, and a detector disposed on top of it, it would be understood that an inverted arrangement would work equally as well. In such an 65 embodiment, the heater would be forced down onto the microfluidic substrate, making contact therewith, and the

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detector would be mounted underneath the substrate, disposed to collect light directed downwards towards it.

In another preferred embodiment (not shown in the FIGs. herein), a cartridge and apparatus are configured so that the read-head does not cover the sample inlets, thereby permitting loading of separate samples while other samples are undergoing PCR thermocycling.

In various embodiments, the apparatus can further include an analysis port. The analysis port can be configured to allow an external sample analyzer to analyze a sample in the microfluidic cartridge. For example, the analysis port can be a hole or window in the apparatus which can accept an optical detection probe that can analyze a sample or progress of PCR in situ in the microfluidic cartridge. In some embodiments, the analysis port can be configured to direct a sample from the microfluidic cartridge to an external sample analyzer; for example, the analysis port can include a conduit in fluid communication with the microfluidic cartridge that directs a liquid sample containing an amplified polynucleotide to a chromatography apparatus, an optical spectrometer, a mass spectrometer, or the like.

In various embodiments, the apparatus can further include one or more force members configured to apply force to at least a portion of a microfluidic cartridge received in the receiving bay. The one or more force members are configured to apply force to thermally couple the at least one heat source to at least a portion of the microfluidic cartridge. The application of force is important to ensure consistent thermal contact between the heater wafer and the PCR reactor and microvalves in the microfluidic cartridge.

The apparatus preferably also includes a processor, comprising microprocessor circuitry, in communication with, for example, the input device and a display, that accepts a user's instructions and controls analysis of samples.

In various embodiments, the apparatus can further include a lid at the receiving bay, the lid being operable to at least partially exclude ambient light from the receiving bay.

In various embodiments, the apparatus can further include at least one input device coupled to the processor, the input device being selected from the group consisting of a keyboard, a touch-sensitive surface, a microphone, and a mouse.

In various embodiments, the apparatus can further include at least one sample identifier coupled to the processor, the sample identifier being selected from an optical scanner such as an optical character reader, a bar code reader, or a radio frequency tag reader. For example, the sample identifier can be a handheld bar code reader.

In various embodiments, the apparatus can further include at least one data storage medium coupled to the processor, the medium selected from: a hard disk drive, an optical disk drive, or one or more removable storage media such as a CD-R, CD-RW, USB-drive, or flash memory card.

In various embodiments, the apparatus can further include at least one output coupled to the processor, the output being selected from a display, a printer, and a speaker, the coupling being either directly through a directly dedicated printer cable, or wirelessly, or via a network connection.

The apparatus further optionally comprises a display that communicates information to a user of the system. Such information includes but is not limited to: the current status of the system; progress of PCR thermocycling; and a warning message in case of malfunction of either system or cartridge. The display is preferably used in conjunction with an external input device as elsewhere described herein, through which a user may communicate instructions to apparatus 100. A suitable input device may further comprise a reader of formatted electronic media, such as, but not limited to, a flash memory

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card, memory stick, USB-stick, CD, or floppy diskette. An input device may further comprise a security feature such as a fingerprint reader, retinal scanner, magnetic strip reader, or bar-code reader, for ensuring that a user of the system is in fact authorized to do so, according to pre-loaded identifying characteristics of authorized users. An input device may additionally—and simultaneously—function as an output device for writing data in connection with sample analysis. For example, if an input device is a reader of formatted electronic media, it may also be a writer of such media. Data that may be written to such media by such a device includes, but is not limited to, environmental information, such as temperature or humidity, pertaining to an analysis, as well as a diagnostic result, and identifying data for the sample in question.

The apparatus may further include a computer network connection that permits extraction of data to a remote location, such as a personal computer, personal digital assistant, or network storage device such as computer server or disk farm. The network connection can be a communications interface selected from the group consisting of: a serial connection, a parallel connection, a wireless network connection, and a wired network connection such as an ethernet or cable connection, wherein the communications interface is in communication with at least the processor. The computer network connection may utilize, e.g., ethernet, firewire, or USB connectivity. The apparatus may further be configured to permit a user to e-mail results of an analysis directly to some other party, such as a healthcare provider, or a diagnostic facility, or a patient.

In various embodiments, there is an associated computer 30 program product includes computer readable instructions thereon for operating the apparatus and for accepting instructions from a user.

In various embodiments, the computer program product can include one or more instructions to cause the system to: 35 output an indicator of the placement of the microfluidic cartridge in the receiving bay; read a sample label or a microfluidic cartridge label; output directions for a user to input a sample identifier; output directions for a user to load a sample transfer member with the PCR-ready sample; output direc- 40 tions for a user to introduce the PCR-ready sample into the microfluidic cartridge; output directions for a user to place the microfluidic cartridge in the receiving bay; output directions for a user to close the lid to operate the force member; output directions for a user to pressurize the PCR-ready sample in 45 the microfluidic cartridge by injecting the PCR-ready sample with a volume of air between about 0.5 mL and about 5 mL; and output status information for sample progress from one or more lanes of the cartridge.

In various embodiments, the computer program product 50 can include one or more instructions to cause the system to: heat the PCR ready-sample under thermal cycling conditions suitable for creating PCR amplicons from the neutralized polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof with at least one probe that 55 is selective for a polynucleotide sequence; independently contact each of the neutralized polynucleotide sample and a negative control polynucleotide with the PCR reagent mixture under thermal cycling conditions suitable for independently creating PCR amplicons of the neutralized polynucle- 60 otide sample and PCR amplicons of the negative control polynucleotide; contact the neutralized polynucleotide sample or a PCR amplicon thereof and the negative control polynucleotide or a PCR amplicon thereof with at least one probe that is selective for a polynucleotide sequence; output a 65 determination of the presence of a polynucleotide sequence in the biological sample, the polynucleotide sequence corre28

sponding to the probe, if the probe is detected in the neutralized polynucleotide sample or a PCR amplicon thereof; and/or output a determination of a contaminated result if the probe is detected in the negative control polynucleotide or a PCR amplicon thereof.

Apparatus 100 may optionally comprise one or more stabilizing feet that cause the body of the device to be elevated above a surface on which system 100 is disposed, thereby permitting ventilation underneath system 100, and also providing a user with an improved ability to lift system 100.

#### **EXAMPLES**

#### Example 1

#### 48 Lane Cartridge

FIG. 23 shows an exemplary 48-lane cartridge for carrying out PCR independently on 48 samples, and with a reaction volume of 10 microliter each. The area occupied by the entire cartridge is approximately 3.5 inches (8.9 cm) by 4.25 inches (10.8 cm). The sample lanes are organized as two groups of 24 each. The adjacent sample lanes in each of the two rows of 24 are spaced apart 4 mm (center-to-center). Trenches between the PCR lanes may be cut in order to isolate the heating of each PCR channel from those adjacent to it. This may be accomplished by etching, milling, controlled cutting, etc., during fabrication of the cartridge.

FIG. **24** shows a heater design used for actuating the 48 lane PCR cartridge of FIG. **23**. The heating of each sample lane can be independently controlled.

#### Example 2

#### PCR Cartridge with Post-PCR Retrieval Capability

Many applications such as genotyping, sequencing, multiple analyte detection (microarray, electrochemical sensing) require post-PCR sample retrieval and subsequent analysis of the retrieved sample in a different instrument. The cartridge of this example, of which a 24 lane embodiment is shown in FIG. 25A, with a sample lane layout illustrated in FIG. 25B, accommodates such a retrieval capability. Each lane in the cartridge of FIG. 25A independently permits sample retrieval. The configuration of the lane of FIG. 25B is different from that of, e.g., FIG. 6 at least because of the presence of 2 gates and the alternative channel from the reactor, via Gate 1, to the inlet. Such features permit effective sample retrieval.

Sample DNA mixed with PCR enzymes is input into a sample lane 2501 through the inlet hole 2502 in the microfluidic network described below. The valves 2506, 2504 (valves 1 and 2) are initially open while the gates 2522, 2520 (gates 1 and 2) are closed, enabling the reaction mix to fill up the PCR reactor 2510 with the excess air venting out through vent hole 1 (label 2514). The valves 1 and 2 are then closed to seal off the reaction mixture. Thermocycling is initiated to conduct the PCR reaction within the PCR reactor. After the reaction is completed, a pipette is mechanically interfaced with the inlet hole 2502 and suction force applied to the pipette. Gates 1 and 2 are opened to enable the reacted sample to exit the PCR reactor and enter the pipette. This controlled opening of the PCR device will also prevent post-PCR contamination of the apparatus in which the cartridge resides as there is minimal exposure of the PCR product with the atmosphere.

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It will be understood that reactions other than PCR can easily be performed in the cartridge of this example.

#### Example 3

#### 12-Lane Cartridge

The 12 channel cartridge of this example is the same basic design that is described and shown in FIG. 3, with the following modifications: the volume of the PCR reactor is increased from 2 µl to 4.5 µl, leading to an increase in the acceptable input volume from 4 µl to 6 µl. Increasing the reaction volume facilitates detection from even dilute samples (wherein the target DNA concentration may be low). In order to detect DNA in a reactor of say 1 microliter volume, there should be a minimum of 1-2 copies of the DNA in the 1 microliter for positive identification, i.e., the concentration should not be less than around 1-2 copies/microliter. Increasing the reaction volume to say 5 microliters will reduce the minimum acceptable starting DNA concentration by 5 fold. The inlet holes are moved a few millimeters away from the edge of the cartridge to allow room for a 2 mm alignment ledge in the cartridge. A similar alignment ledge is also included on the other edge of the cartridge. The alignment ledge permits the cartridges to be 25 stacked during storage (or within a multi-cartridge springloader) without the hydrophobic vent of one cartridge coming into contact with a surface of an adjacent cartridge.

#### Example 4

#### 24-Lane Cartridge

This 24-lane cartridge has two rows of 12 sample lanes. Each lane has: a liquid inlet port, that interfaces with a disposable pipette; a 4 microliter PCR reaction chamber (1.5 mm wide, 300 microns deep and approximately 10 mm long), and two microvalves on either side of the PCR reactor and outlet vent. Microvalves are normally open, and close the channel on actuation. The outlet holes enable extra liquid (~1  $\mu$ l) to be 40 contained in the fluidic channel in case more than 6  $\mu$ l of fluid is dispensed into the cartridge. Thus, the cartridge of this example does not require a bubble vent as it will be used in an automated PCR machine having a reliable, precision liquid dispenser.

The inlet holes of the cartridge of this example are made conical in shape and have a diameter of 3-6 mm at the top to ensure that pipette tips can be easily landed by an automated fluid dispensing head into the conical hole, with some tolerance. There is also an optional raised annulus around the top 50 of the holes. Once the pipette tip lands within the cone, the conical shape guides the pipette and mechanically seals to provide error free dispensing into, or withdrawal of fluid from, the cartridge. The bigger the holes, the better it is to align with the pipette, however, given the opposing need to 55 maximize the number of inlet ports within the width of the cartridge as well as to maintain the pitch between holes compatible with the inter-pipette distance, the holes cannot be too big. In this design, the inter-pipette tip distance is 18 mm and the distance between the loading holes in the cartridge is 6 60 mm. So lanes 1, 4, 7, 11 are pipetted into during one dispensing operation that utilizes four pipette tips; lanes 2, 5, 8 and 12 in the next, and so on and so forth.

The height of the conical holes is kept lower than the height of the ledges on the edges of the cartridge to ensure the 65 cartridges can be stacked on the ledges. The ledges on the two long edges of the cartridge enable stacking of the cartridges

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with minimal surface contact between two stacked cartridges and also help guide the cartridge into the reader from a spring-loader, where used.

#### Example 5

#### 12-Lane Cartridge

This 12-lane cartridge has 12 sample lanes in parallel, as shown in FIG. 1. Each lane has: a liquid inlet port that interfaces with a disposable pipette; a bubble vent; a PCR reaction chamber, and two microvalves on either side of the PCR reactor and outlet vent. Microvalves are normally open, and close the channel on actuation. The reaction volume is in the range 1-10  $\mu$ l so that the number of copies of DNA will be sufficient for detection. Such a volume also permits the PCR reaction volume to be similar to release volume from a sample preparation procedure.

#### Example 6

#### Kit

FIG. 26 shows a representative sample kit 2610 that includes a microfluidic cartridge 2612 with a barcode label 2632, and one or more sample containers 2614 each also optionally having a barcode label.

FIG. 27 shows that one or more components of the sample kit, for example, microfluidic cartridge 2612, can be packaged in a sealed pouch 2624. The pouch can be hermetically sealed with an inert gas such as argon, nitrogen, or others.

The barcode labels of both cartridge and sample container can be read with a bar code reader prior to use.

#### Example 7

Apparatus and Process for Wax Loading of Valves

Exemplary Wax-Deposition Process

Deposition of wax in valves of the microfluidic network, as at step 1804 of FIG. 18 may be carried out with the exemplary equipment shown in FIGS. 28A and 28B. The DispenseJet Series DJ-9000 (available from Asymtek, Carlsbad, Calif.) is a non-contact dispenser suitable for this purpose that provides rapid delivery and high-precision volumetric control for various fluids, including surface mount adhesive, underfill, encapsulants, conformal coating, UV adhesives, and silver epoxy. The DJ-9000 jets in tight spaces as small as 200 micrometers and creates fillet wet-out widths as small as 300 micrometers on the dispensed side of a substrate such as a die. It dispenses fluid either as discrete dots or a rapid succession of dots to form a 100-micron (4 mil) diameter stream of fluid from the nozzle. It is fully compatible with other commercially available dispensing systems such as the Asymtek Century C-718/C-720, Millennium M-2000, and Axiom X-1000 Series Dispensing Systems.

A DJ-9000 is manufactured under quality control standards that aim to provide precise and reliable performance. Representative specifications of the apparatus are as follows.

Characteristic	Specification
Size	Width: 35 mm Height: 110 mm Depth: 100 mm

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Characteristic	Specification
Weight	400 grams - dry
Feed Tube Assembly	Nylon -Fitting
	Polyurethane - Tube
Fluid Chamber	Type 303 Stainless Steel
Seat and Nozzle	300/400 Series S/S, Carbide
Needle Assembly	52100 Bearing Steel - Shaft
	Hard Chrome Plate
	Carbide - Tip
Fluid Seal	PEEK/Stainless Steel
Fluid Chamber 0-Ring	Ethylene Propylene
Jet Body	6061-T6 Aluminum
	Nickel Plated
Needle Assembly Bearings	PEEK
Thermal Control Body	6061-T6 Aluminum
	Nickel Plated
Reservoir Holder	Acetyl
Reservoir Size	5, 10, or 30 cc (0.17, 0.34,
	or 1.0 oz)
Feed Tube Assembly Fitting	Female Luer per ANSI/HIMA
	MD70.1-1983
Maximum Cycle Frequency	200 Hz.
Minimum Valve Air Pressure	5.5 bar (80 psi)
Operating Noise Level	70 db*
Solenoid	24 VDC, 12.7 Watts
Thermal Control Heater	24 VDC, 14.7 Watts, 40 ohms
Thermal Control RTD	100 ohm, platinum
Maximum Heater Set Point	80° C.

<sup>\*</sup>At Maximum Cycle Rate

An exploded view of this apparatus is shown in FIG. **28**B. Theory of Operation of DJ-9000

The DJ-9000 has a normally closed, air-actuated, spring-return mechanism, which uses momentum transfer principles to expel precise volumes of material. Pressurized air is regulated by a high-speed solenoid to retract a needle assembly from the seat. Fluid, fed into the fluid chamber, flows over the seat. When the air is exhausted, the needle travels rapidly to the closed position, displacing fluid through the seat and nozzle in the form of a droplet. Multiple droplets fired in succession can be used to form larger dispense volumes and lines when combined with the motion of a dispenser robot.

The equipment has various adjustable features: The following features affect performance of the DJ-9000 and are typically adjusted to fit specific process conditions.

Fluid Pressure should be set so that fluid fills to the seat, but should not be influential in pushing the fluid through the seat 45 and nozzle. In general, higher fluid pressure results in a larger volume of material jetted.

The Stroke Adjustment controls the travel distance of the Needle Assembly. The control is turned counterclockwise to increase needle assembly travel, or turned clockwise to 50 decrease travel. An increase of travel distance will often result in a larger volume of material jetted.

The Solenoid Valve controls the valve operation. When energized, it allows air in the jet air chamber to compress a spring and thereby raise the Needle Assembly. When deenergized, the air is released and the spring forces the piston down so that the needle tip contacts the seat.

The seat and nozzle geometry are typically the main factors controlling dispensed material volume. The seat and nozzle size are determined based on the application and fluid properties. Other parameters are adjusted in accordance with seat and nozzle choices. Available seat and nozzle sizes are listed in the table hereinbelow.

Thermal Control Assembly: Fluid temperature often influences fluid viscosity and flow characteristics. The DJ-9000 is equipped with a Thermal Control Assembly that assures a constant fluid temperature.

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Dot and Line Parameters: In addition to the DJ-9000 hardware configuration and settings, Dot and Line Parameters are set in a software program (referred to as FmNT) to control the size and quality of dots and lines dispensed.

#### Example 7

#### 24-Lane Cartridge

FIGS. 29A-29C show an exemplary 24-lane cartridge having three layers in its construction in which there is no hydrophobic membrane, and no thermally compliant layer. The three layers are a laminate 2922, a microfluidic substrate 2924, and a label 2926. A typical reaction vol. is 4.5 µl in each lane from 2 rows of 12 lanes. No bubble-removal vents are utilized and instead of a hydrophobic end vent, there is just a hole. This is consistent with use of an accurate pipetting system. There is no thermally compliant/conductive layer for situations where enough pressure can be reliably applied to the cartridge that effective thermal contact with the microfluidic substrate can be made without requiring the additional layer. The absence of two layers from the construction saves manufacturing costs.

#### Example 8

#### 96-Lane Cartridge

FIGS. 30A-D show aspects of a 96-lane cartridge design, including complementary heater configurations. (FIG. 30A shows cartridge design; 30B shows heater design in a single metal layer; 30C shows individual PCR channels overlaid with heater configurations; 30D shows individual PCR lanes.) In the embodiment shown, liquid sample is loaded without air bubbles as the lanes do not have any vents. Two or more Mux can be utilized for controlling all 96 PCR channels.

Such an arrangement lends itself to whole area imaging (e.g., by a CCD) for detection instead of optical based methods using diodes and lenses.

#### Example 9

#### Real-Time PCR

FIG. 31 shows a trace of real-time PCR carried out on multiple samples in parallel with an apparatus and microfluidic network as described herein. The PCR curves are standard plots that are representative of fluorescence from 12 different PCR lanes as a function of cycle number.

The foregoing description is intended to illustrate various aspects of the present technology. It is not intended that the examples presented herein limit the scope of the present technology. The technology now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

What is claimed:

- 1. A microfluidic cartridge comprising a microfluidic substrate layer, the microfluidic substrate layer comprising:
  - a first reaction chamber;
  - a second reaction chamber;
  - a first inlet port for introducing a first sample onto the microfluidic substrate layer, the first inlet port formed in a surface of the microfluidic substrate layer and in fluid communication with the first reaction chamber;
  - a second inlet port for introducing a second sample onto the microfluidic substrate layer, the second inlet port spaced

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- apart from the first inlet port on the surface of the microfluidic substrate layer, the second inlet port in fluid communication with the second reaction chamber;
- a first outlet, in fluid communication with the first reaction chamber;
- a second outlet, in fluid communication with the second reaction chamber;
- a first set of microfluidic valves configured to isolate the first reaction chamber from the first inlet port and the first outlet; and
- a second set of microfluidic valves configured to isolate the second reaction chamber from the second inlet port and the second outlet independent of the isolation of the first reaction chamber by the first set of microfluidic valves,
- wherein the isolation effected by the first and the second set of microfluidic valves prevents movement of fluid into and out of the first and the second reaction chambers, wherein the first set of microfluidic valves comprises a first microfluidic valve spatially separated from the first inlet port and a second microfluidic valve spatially separated from the first outlet, and wherein the second set of microfluidic valves comprises a first microfluidic valve spatially separated from the second microfluidic valve spatially separated from the second microfluidic valve spatially separated from the second outlet, and wherein each of the first and second 25 reaction chambers, the first and second inlet ports, the first and second outlets, and the first and second sets of microfluidic valves are all formed in the microfluidic substrate layer.
- 2. The microfluidic cartridge of claim 1, wherein the first 30 reaction chamber and the second reaction chamber are configured to amplify one or more polynucleotides independently of the other chamber.
- 3. The microfluidic cartridge of claim 1, wherein the first outlet comprises a first vent and the second outlet comprises 35 a second vent.
- **4**. The microfluidic cartridge of claim **1**, wherein the first inlet port and the second inlet port are configured to accept a sample from a pipette tip.
- 5. The microfluidic cartridge of claim 1, configured to carry 40 out real-time PCR in at least one of the reaction chambers.
- 6. The microfluidic cartridge of claim 1, wherein the first inlet port and the second inlet port are spaced apart from one another to permit simultaneous loading from a multiple-pipette head dispenser.
- 7. The microfluidic cartridge of claim 1, wherein the first set of microfluidic valves and the second set of microfluidic valves comprise a temperature responsive substance that melts upon heating and seals the first and the second reaction chambers.
- 8. The microfluidic substrate of claim 1, wherein the first and second reaction chambers and the first and second sets of microfluidic valves are formed in a first side of the microfluidic substrate layer, and wherein the first and second inlet ports and the first and second outlets are formed in a second 55 side of the microfluidic substrate layer opposite the first side.
- **9.** A method of carrying out PCR independently on a plurality of polynucleotide-containing samples, the method comprising:
  - introducing the plurality of samples into the microfluidic 60 cartridge of claim 1, wherein the cartridge has a plurality of reaction chambers comprising the first reaction chamber and the second reaction chamber, the plurality of reaction chambers configured to permit thermal cycling of the plurality of samples independently of one another; 65 moving the plurality of samples into the respective plurality of reaction chambers;

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- isolating the plurality of reaction chambers; and amplifying polynucleotides contained with the plurality of samples, by application of successive heating and cooling cycles to the reaction chambers.
- 10. A microfluidic substrate, comprising:
- a plurality of sample lanes, wherein each of the plurality of sample lanes comprises a microfluidic network having, in fluid communication with one another:
  - a first valve and a second valve;
  - a first channel leading from the inlet, via the first valve, to a reaction chamber; and
  - a second channel leading from the reaction chamber, via the second valve, to a vent,
  - wherein the first valve and the second valve are configured to isolate the reaction chamber from the inlet and the vent to prevent movement of fluid into or out of the reaction chamber, wherein the first valve is spatially separated from the inlet and the second valve is spatially separated from the vent, wherein the reaction chamber, the first channel, and the second channel are formed in a first side of the microfluidic substrate, wherein the inlet and the vent are formed in a second side of the microfluidic substrate opposite the first side, and wherein the first valve in each of the plurality of sample lanes is operated independently of any other first valve.
- 11. The microfluidic substrate of claim 10, additionally comprising:
  - a third channel leading from the inlet to the reaction chamber, wherein a gate is positioned in the third channel, and wherein the gate is configured to open the third channel to permit material from the reaction chamber to be removed via the inlet.
- 12. The microfluidic substrate of claim 10, wherein each of the plurality of sample lanes is configured to amplify one or more polynucleotides independently of the other lanes.
- 13. The microfluidic substrate of claim 10, wherein each of the plurality of sample lanes further compiles a bubble vent.
- **14**. The microfluidic substrate of claim **10**, wherein the inlet is configured to accept sample from a pipette tip.
- 15. The microfluidic substrate of claim 10, configured to carry out real-time PCR in at least one of the reaction chambers
- 16. The microfluidic substrate of claim 10, wherein the inlets of the respective plurality of sample lanes are spaced apart from one another to permit simultaneous loading from a multiple-pipette head dispenser.
- 17. The microfluidic substrate of claim 10, wherein the first and second valves comprise a temperature responsive substance that melts upon heating and seals the reaction chamber.
- **18**. The microfluidic substrate of claim **10**, wherein the second valve in each of the plurality of sample lanes is operated independently of any other second valve.
- 19. A microfluidic cartridge comprising the microfluidic substrate of claim 10.
- 20. The microfluidic cartridge of claim 19, further comprising a registration member that ensures that the cartridge is received by a complementary diagnostic apparatus in a single orientation.
- 21. The microfluidic cartridge of claim 19, wherein each of the microfluidic networks, including the reaction chamber, the inlet, and the valves for isolating the reaction chamber, is defined in a single substrate.

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22. The microfluidic cartridge of claim 21, wherein the substrate is a rigid substrate and impervious to air or liquid, and entry or exit of air or liquid during operation of the cartridge is only possible through the inlet or a vent.

\* \* \*

# EXHIBIT 7

#### Filter News

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BD Diagnostic Systems and HandyLab, Inc. Announce **Exclusive Collaboration to** Commercialize Molecular

Published: May 18, 2009



Assays

FRANKLIN LAKES, N.J. and ANN ARBOR, Mich., May 18 /PRNewswire-FirstCall/ -- BD Diagnostics, a segment of BD (Becton, Dickinson and Company) (NYSE: BDX -News), and HandyLab, Inc. announced today that they have entered into an exclusive agreement for BD to commercialize its molecular assays on a new BD MAX(TM) system, an automated molecular diagnostic testing platform in development using HandyLab's recently launched Jaguar instrument. BD will focus initially on its BD GeneOhm(TM) line of molecular assays to detect major pathogens associated with healthcare-associated infections (HAIs).

"This collaboration further demonstrates BD's commitment to address underappreciated healthcare needs," said Philippe Jacon, President, BD Diagnostics -Diagnostic Systems. "As our customers expand their HAI and sexually transmitted infection testing programs, they are seeking simple, flexible and fully automated systems to meet their increasing demands. Our new BD MAX system will be a good strategic fit with our current platforms and those under development."

Released by HandyLab in November 2008, the Jaguar system is the first fully integrated bench-top molecular diagnostic system to provide hands-off operation. The system incorporates clinical sample preparation, nucleic acid extraction, and microfluidic real-time

polymerase chain reaction (PCR) amplification and detection. The self-contained workstation is designed to accommodate on-demand and batch workflows. It requires minimal laboratory space and minimal skill levels to generate up to 24 real-time PCR results in under two hours.

"This exclusive collaboration with BD represents an important step forward in expanding the utility of the Jaguar system," said Jeff Williams, President and CEO, HandyLab. "BD is a global leader in molecular diagnostic testing for infectious diseases. Together we plan to provide diagnostic laboratories with a broad molecular test menu on one of the industry's most advanced automation platform."

BD is a leader in rapid molecular test methods to help detect major HAI-associated pathogens including methicillin-resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, vancomycin-resistant enterococcus and toxigenic Clostridium difficile. The BD GeneOhm(TM) MRSA assay has been used to test over 4 million patients worldwide for carriage of the potentially lethal MRSA bacteria. BD is also a leader in molecular testing for Chlamydia and gonorrhea with its BD ProbeTec(TM) Chlamydia trachomatis (CT) Amplified DNA Assay and the BD ProbeTec(TM) Neisseria gonorrhoeae (GC) Amplified DNA Assay.

About HandyLab, Inc.

HandyLab is dedicated to the development, manufacture and sale of novel molecular diagnostic products. HandyLab's proprietary platform reduces the time, cost and complexity of testing while improving the quality of results. Using patented real time microfluidic PCR technology, HandyLab's products are positioned to decentralize nucleic acid testing. HandyLab is an Ann Arbor, Michigan based company. Additional information about the company can be found on the internet at www.handylab.com, or by calling 1-866-Handy Lab (1-866-426-3952).

About BD

BD is a leading global medical technology company that develops, manufactures and sells medical devices, instrument systems and reagents. The Company is dedicated to improving people's health throughout the world. BD is focused on improving drug delivery, enhancing the quality and speed of diagnosing infectious diseases and cancers, and advancing research, discovery and production of new drugs and vaccines. BD's capabilities are instrumental in combating many of the world's most pressing diseases. Founded in 1897 and headquartered in Franklin Lakes, New Jersey, BD employs approximately 28,000 people in approximately 50 countries throughout the world. The Company serves healthcare institutions, life science researchers, clinical laboratories, the pharmaceutical industry and the general public. For more information, please visit www.bd.com.

This press release contains certain estimates and other forward-looking statements (as defined under Federal securities laws) regarding BD's performance, including future performance, products or other events or developments that BD expects to occur or anticipates occurring in the future. All such statements are based upon current expectations of BD and involve a number of business risks and uncertainties. Actual results could vary materially from anticipated results described, implied or projected in any forward-looking statement. Factors that could cause actual results to vary materially from any forward-looking statement include, but are not limited to: competitive factors; pricing and market share pressures; difficulties inherent in product development and delays in product introductions; changes in regional, national or foreign economic conditions; increases in energy costs and their effect on, among other things, the cost of producing BD's products; fluctuations in costs and availability of raw materials and in BD's ability to

maintain favorable supplier arrangements and relationships; changes in healthcare or other governmental regulation; as well as other factors discussed in this press release and in BD's filings with the Securities and Exchange Commission. We do not intend to update any forward-looking statements to reflect events or circumstances after the date hereof except as required by applicable laws or regulations.



■ Back to news

# EXHIBIT 8

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Posted on Fri, Oct 23, 2009: 11:32 a.m.

### Ann Arbor's HandyLab acquired by medical device firm in \$300 million deal

By Nathan Bomey

Ann Arbor medical devices firm HandyLab (http://www.handylab.com/), a promising technology company often held up as a bright spot in the local economy, was acquired today (http://www.prnewswire.com/news-releases/bdannounces-agreement-to-acquire-handylab-inc-65757862.html) by New Jersey-based medical devices manufacturer Becton, Dickinson and Company (http://www.bd.com/). Sources said the deal is worth close to \$300 million, although the companies wouldn't release details of the transaction.

HandyLab, a University of Michigan (http://www.umich.edu/) spinoff that employs about 60 people in Pittsfield Township, will maintain its local operation and the company's management, BD spokeswoman Colleen White



HandyLab CEO Jeff Williams

BD, which has some 29,000 employees in 50 countries, reports revenue of about \$7.2 billion (http://finance.yahoo.com/q/ks?s=BDX). The firm, which has a Detroit facility (http://maps.google.com/maps?oe=utf-8&rls=org.mozilla:en-US:official&client=firefoxa&um=1&ie=UTF-

8&q=bectin+dickson+detroit+mi&fb=1&ql=us&hq=bectin+dickson&hnear=detroit+mi&cid=0,0,8341574114903883087&ei=vNbhSum2H expects to finish the acquisition later this year. Officials declined to release terms of the deal.

HandyLab CEO Jeff Williams played an instrumental role in developing the company - founded by U-M grads Kalyan Handique (http://www.handylab.com/handique.html), known as "Handy," and Sundaresh Brahmasandra (http://www.handylab.com/brahmasandra.html) - since joining it in 2004. He said the deal would allow HandyLab to expand distribution of its lead product.

"They can help us expand the distribution very rapidly," Williams said. "It's a good outcome for our shareholders, for our employees and for our customers."

The acquisition marks a significant victory for the Michigan venture capital Kalyan Kandique community. Ann Arbor VC firms Ardesta (http://www.ardesta.com/), EDF Ventures (http://www.edfvc.com/), Arboretum Ventures (http://www.arboretumvc.com/) and the student-run Wolverine Venture Fund (http://www.zli.bus.umich.edu/wvf/) financed the company before outside investors came along.

"It's a big win for everybody," said Jan Garfinkle, managing director of Arboretum, which funded the company in 2004 and owns a 10 percent stake. "The Michigan venture capital funds were absolutely 100 percent key to the financing of this company."

HandyLab chairman Rick Snyder, CEO of Ardesta, funded HandyLab, shortly after EDF and the Wolverine Venture Fund helped the company get on its feet in 1999. He wouldn't disclose the details of Ardesta's ownership stake. The deal comes 12 months after Ann Arbor-based HealthMedia (http://www.healthmedia.com/), which had funds from Ardesta and Arboretum, was acquired by Johnson & Johnson (http://www.jnj.com/).

Snyder, also a Republican gubernatorial candidate, said HandyLab's Michigan venture capital funding was crucial to its inception.

HandyLab in 2008 acquired \$19.2 million (http://www.crainsdetroit.com/article/20080728/SUB/807280319) in venture capital, adding to some \$40 million previously secured.

"It never would have been created" without the VC funding, Snyder said. "If it would've gotten going, it probably would have moved. It's an outstanding opportunity of the success that we can have in Michigan."



HandyLab co-founder



EDF Ventures partner Mary Campbell



identify infections, could eventually lead to additional manufacturing,

technology and sales jobs in Ann Arbor.
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"It is going to revolutionize how infections are detected, both the speed at which they're detected and the accuracy," Garfinkle said.

The acquisition adds to BD's growing molecular diagnostics portfolio.

"We have a big presence in molecular diagnostics and they're bringing a flexible, automated platform that adds" to BD's technology, White said.

Stephen Rapundalo, executive director of Ann Arbor-based MichBio (http://michbio.org/), the state's life sciences association, said the diagnostic devices industry represents opportunity for Michigan.

"I think that's a subsector of the industry is definitely growing here," Rapundalo said. "I hope that BD will recognize the talent that's here in the area and make the correct decision to keep HandyLab and expand the company here."

Williams said BD is incentivized to keep HandyLab in Ann Arbor because of the company's talent.

"A lot of what they're buying is corporate knowledge that's invested in our people," Williams said. "It's pretty complex technology that's quite specialized. We've over the years built up a lot of capability here in Ann Arbor that really has to stay put."

The acquisition comes seven months after HandyLab expanded its footprint in Pittsfield Township by taking over (http://www.mlive.com/business/ann-arbor/index.ssf/2009/03/handylab\_expands\_footprint\_in.html) 4,500 square feet of space in its South State Street building. The move means HandyLab now leases the entire 22,000-square-foot building at 5230 S. State St.

Elizabeth Parkinson, director of marketing and public relations for economic development group Ann Arbor SPARK (http://www.annarborspark.org/), said HandyLab's acquisition shows the power of the local venture capital industry and entrepreneurial community.

"For the entrepreneurial ecosystem in the region and in the state, it's very, very positive," Parkinson said.



Arboretum Ventures managing director Jan Garfinkle



Ardesta CEO Rick Snyder

AnnArbor.com Business News Director Paula Gardner contributed to this report.

Contact AnnArbor.com's Nathan Bomey at (734) 623-2587 or nathanbomey@annarbor.com (mailto:nathanbomey@annarbor.com). You can also follow him on Twitter (http://twitter.com/NathanBomey).

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# EXHIBIT 9

Jurisdiction	Patent Number	Patent Title	Exemplary Claim	Expiration
USA	8,088,616	Heater unit for microfluidic diagnostic system	A heater substrate, comprising:  a plurality of groups of resistive heaters, and at least one temperature sensor per group of heaters, wherein a portion of the substrate is removed from around the resistive heaters to reduce the effective thermal mass adjacent to the heater group; and control circuitry for supplying electric current to the plurality of groups of resistive heaters at selected intervals, wherein the substrate has a surface configured to make thermal contact with a microfluidic cartridge having a plurality of PCR reaction chambers, and to deliver heat from the plurality of groups of resistive heaters to regions of the cartridge, such that each of the groups of resistive heaters delivers heat to a select PCR reaction chamber to perform a reaction, wherein the heat delivery from each group of resistive heaters is controlled by sensing temperature using the at least one temperature	Mar. 2030
USA	8,324,372	Polynucleotide capture materials, and methods of using same	sensor of the group.  18. A method for concentrating RNA from a sample containing polymerase chain reaction inhibitors, the method comprising:     contacting between 500 μl and 1 ml of the sample with a plurality of RNA binding particles, wherein the binding particles retain the RNA in the sample as compared to the polymerase chain reaction inhibitors;     magnetically compacting the plurality of binding particles having the one or more polynucleotides bound thereto into an effective volume between 50 nanoliters and 5 microliters; and	Feb. 2029
USA	8,415,103	Microfluidic cartridge	A method of carrying out amplification independently on a plurality of polynucleotide-containing samples, the method comprising:     introducing the plurality of samples separately into a microfluidic cartridge;     isolating the samples in the microfluidic cartridge;     placing the microfluidic cartridge in thermal communication with an array of independent heaters; and     amplifying polynucleotides in the plurality of samples by independent application of successive temperature cycles to each sample.	Nov. 2027

USA	7,998,708	Microfluidic system for amplifying and detecting polynucleotides in parallel	An apparatus, comprising:  a multi-lane microfluidic cartridge, each lane comprising a PCR reaction zone;  a receiving bay configured to receive the microfluidic cartridge;  each PCR reaction zone comprising a separately controllable heat source thermally  coupled thereto, wherein the heat source maintains a substantially uniform temperature throughout the PCR reaction zone and thermal cycles the PCR reaction zone to carry out  PCR on a polynucleotide-containing sample in the PCR reaction zone;  a detector configured to detect the presence of an amplification product in the respective PCR reaction zone; and  a processor coupled to the detector and the heat source, configured to control heating of one or more PCR reaction zones by the heat sources.	Mar. 2027
	8,323,900	Microfluidic system for amplifying and detecting polynucleotides in parallel	1. An apparatus, comprising: a plurality of multi-lane microfluidic cartridges, each lane comprising a PCR reaction zone; a plurality of receiving bays, each receiving bay configured to receive one of the plurality of microfluidic cartridges; each PCR reaction zone comprising a separately controllable heat source thermally coupled thereto, wherein the heat source thermal cycles the PCR reaction zone to carry out PCR on a polynucleotide-containing sample in the PCR reaction zone and maintains a substantially uniform temperature throughout the PCR reaction zone during each cycle; a detector configured to detect the presence of an amplification product in one or more PCR reaction zones; and a processor coupled to the detector and the heat sources, configured to control heating of one or more PCR reaction zones by the heat sources.  20. A method of carrying out PCR on a plurality of samples, the method comprising: introducing the plurality of samples into a plurality of multi-lane microfluidic cartridges, wherein each lane comprises a PCR reaction zone configured to permit thermal cycling of a sample independently of the other samples; moving the plurality of samples into the respective plurality of PCR reaction zones; and amplifying polynucleotides contained with the plurality of samples in the plurality of PCR reaction zones while thermal cycling the PCR reaction zones and maintaining a substantially uniform temperature throughout each PCR reaction zone during each cycle, at least one PCR reaction zone separately thermally controllable from another PCR reaction zone. & 1 more indep claim	Mar. 2027

USA	9,040,288	Integrated system for processing microfluidic samples, and method of using the same	1. A diagnostic apparatus, comprising:     a first module configured to extract nucleic acid simultaneously from a plurality of nucleic acid-containing samples, wherein the first module comprises:     one or more racks, each configured to accept the plurality of samples and a corresponding number of holders, wherein each holder comprises a process chamber, a waste chamber, one or more pipette tips, and one or more receptacles, wherein the one or more receptacles contain respectively sufficient quantities of one or more reagents for carrying out extraction of nucleic acid from a sample;     a magnetic separator configured to move adjacent to the one or more racks in order to apply a magnetic force to contents of the process chamber of each holder;     a heater assembly configured to independently heat each of the process chambers; and a liquid dispenser configured to carry out fluid transfer operations on two or more holders simultaneously; and     a second module configured to simultaneously amplify the nucleic acid extracted from the plurality of samples, wherein the second module comprises: one or more bays, each bay comprising at least one heat source and configured to receive a microfluidic cartridge, wherein the cartridge is configured to separately accept and to separately amplify the nucleic acid extracted from multiple samples; and one or more detection systems.	Mar. 2027
	9,080,207	Microfluidic system for amplifying and detecting polynucleotides in parallel	1. An apparatus, comprising:     a plurality of multi-lane microfluidic cartridges, each lane comprising a reaction zone;     a plurality of receiving bays, each receiving bay configured to receive one of the     plurality of microfluidic cartridges;     each reaction zone comprising multiple separately controllable heaters thermally     coupled thereto, the heaters configured to simultaneously and uniformly heat the     reaction zone to carry out nucleic acid amplification on a polynucleotide-containing     sample in the reaction zone;     a detector configured to detect the presence of an amplification product in one or     more reaction zones; and     a processor coupled to the detector and the heaters, configured to control heating of     one or more reaction zones by the heaters.	Mar. 2027

USA	8,273,308	Moving microdroplets in a microfluidic device	A device, comprising:  a microfluidic process module;  a computer-controlled heat source; and  a detector;  wherein the microfluidic process module comprises:  a zone configured to receive a sample and perform amplification of the sample;  a first valve upstream of the zone;  a second valve downstream of the zone; and  a vent separated from the first valve by the second valve;  a controller programmed to close the first and second valves to prevent gas and liquid from flowing into or out of the zone when amplification of the sample occurs in the zone, wherein the only ingress to and egress from the zone is through the first and second valves; wherein the computer-controlled heat source is in thermal contact with the zone; and wherein the detector is configured to identify one or more polynucleotides within the zone.	
USA	8,703,069	Moving microdroplets in a microfluidic device	A method of amplifying a nucleic acid-containing sample within a microfluidic device, the method comprising: moving the sample from an upstream channel of the microfluidic device into a DNA manipulation module located downstream of the upstream channel, the DNA manipulation module including a DNA manipulation zone configured to perform amplification of the sample, a first valve disposed upstream of the DNA manipulation zone, and a second valve disposed downstream of the DNA manipulation zone, the only ingress to and egress from the DNA manipulation zone being through the first valve and the second valve; receiving the sample in the DNA manipulation zone; closing the first valve and the second valve such that gas and liquid are prevented from flowing into or out of the DNA manipulation zone; and thermal cycling the sample in the DNA manipulation zone.	Mar. 2021

EP	EP2001990B1	INTEGRATED SYSTEM FOR PROCESSING MICROFLUIDIC SAMPLES, AND METHOD OF USING SAME	1. An apparatus (981), comprising:     a receiving bay (992, 2014) configured to receive an insertable multi-lane microfluidic cartridge (994), each lane comprising a PCR reaction zone (1001) configured to accept a polynucleotide-containing sample (996);     a plurality of sets of heaters, each set of heaters thermally coupled to a PCR reaction zone in the multi-lane cartridge, each set of heaters comprising a plurality of heaters (1003, 1005, 1007, 1009) together configured to cyclically heat the PCR reaction zone under thermal cycling conditions, the plurality of heaters further configured to maintain a substantially uniform temperature throughout the PCR reaction zone during each phase of the thermal cycling a detector (999, 2009) configured to detect the presence of one or more amplified polynucleotides in the multi-lane cartridge; and     a processor (980) configured to independently control each heater in order to cyclically heat each PCR reaction zone of the multi-lane cartridge, the processor further configured to perform independent PCR reactions on polynucleotide-containing samples in the PCR reaction zones of the multi-lane cartridge.	Mar. 2027
EP	EP3088083B1	METHOD OF PERFORMING PCR WITH A MULT-ILANE CARTRIDGE	1. A method, comprising: inserting a multi-lane microfluidic cartridge (994) into a receiving bay (992) of an apparatus (981), wherein each lane of the multi-lane microfluidic cartridge (994) comprises a PCR reaction zone (1001) configured to accept a polynucleotide containing sample, and wherein the apparatus (981) comprises a plurality of sets of heaters, each set of heaters coupled to a PCR reaction zone (1001) in the multi-lane cartridge, each set of heaters comprising a plurality of heaters (1003, 1005, 1007, 1009) together configured to maintain a substantially uniform temperature throughout a PCR reaction zone (1001); performing independent PCR reactions on polynucleotide-containing samples in the PCR reaction zones (1001) of the multi-lane cartridge (994), wherein performing the independent PCR reactions comprises independently controlling each heater (1003, 1005, 1007, 1009), using a processor (980) of the apparatus (981), in order to cyclically heat each PCR reaction zone (1001) under thermal cycling conditions, maintaining a substantially uniform temperature throughout each PCR reaction zone (1001) during each phase of the thermal cycling; and detecting, using a detector (999) of the apparatus (981), the presence of one or more amplified polynucleotides in the multi-lane cartridge (994).	Mar. 2027

Jurisdiction	Patent Application	Patent Title	Exemplary Claim	Expiration
USA	16/124,672	INTEGRATED APPARATUS FOR PERFORMING NUCLEIC ACID EXTRACTION AND DIAGNOSTIC TESTING ON MULTIPLE BIOLOGICAL SAMPLES	A preparatory apparatus comprising:     a rack;     a device configured to be removably received in the rack, the device comprising a process chamber, wherein the process chamber is configured to receive magnetic particles capable of binding to one or more biomolecules;     a magnetic separator configured to apply a magnetic force to the process chamber of the device received in the rack, wherein the magnetic force is capable of moving the magnetic particles relative to an innter surface of the process chamber;     a heater assembly configured to heat the process chamber of the device received in the rack, wherein the magnetic separator is configured to operate in conjunction with the heater assembly; and     a liquid dispenser configured to carry out fluid transfer operations relative to the device.	Nov. 2027
USA	13/341,477	HEATER UNIT FOR MICROFLUIDIC DIAGNOSTIC SYSTEM	1. A system, comprising:  a microfluidic cartridge comprising a plurality of amplification reaction chambers; and a substrate comprising: a plurality of groups of resistive heaters, each group of the plurality of resistive heaters comprising two or more resistive heaters, wherein each of the two or more resistive heaters is separately and independently controlled by control circuitry programmed to separately and independently control each of the two or more resistive heaters; at least one temperature sensor for each group of the plurality of resistive heaters; wherein each group of the plurality of resistive heaters is positioned in a different region of the substrate; and control circuitry programmed to sepearately and independently supply electric for supplying electric current to each of the two or more resistive heaters at selected intervals, wherein a flat area of the substrate is configured to removable receive and make thermal contact with the microfluidic cartridge such that each group of the polurality of resistive heaters is configured to deliver heat to a single amplification reaction chamber to perform a reaction, wherein the heat delivery from each group of the plurality of resistive heaters is controlled by sensing temperature using the at least one temperature sensor of each group, and 3 other independent claims	Mar. 2027

USA	14/719,692	INTEGRATED SYSTEM FOR PROCESSING MICROFLUIDIC SAMPLES, AND METHOD OF USING SAME	50. An apparatus comprising:  a receiving bay comprising a planar surface configured to receive a multi-lane microfluidic cartridge, the multi-lane microfluidic cartridge comprising a first lane configured to accept a first sample, the first lane comprising a first PCR reaction zone and a second lane configured to accept a second sample, the second lane comprising a second PCR reaction zone;  a processor;  a memory in communication with the processor;  the receiving bay comprising a first heater set thermally coupled to the first PCR reaction zone and a second heater set thermally coupled to the second PCR reaction zone when the multi-lane microfluidic cartridge is in thermal communication with the planar surface of the receiving bay, each heater set fixed in position in the receiving bay, each heater set fixed in position relative to the planar surface of the receiving bay during heating, each heater set comprising four heaters;  the memory comprising instructions that direct the processor to independently control the temperature of each of the four heaters of the first heater set to cyclically heat the four heaters in a series of heating phases, wherein each heating phase comprises each of the four heaters of the first heater set to maintain a substantially uniform temperature in the first PCR reaction zone during each heating phase; and the memory comprising instructions that direct the processor to perform independent reactions on the first sample in the first PCR reaction zone and the second sample in the second PCR reaction zone.	Mar. 2027
USA	14/796,239	MICROFLUIDIC SYSTEM FOR AMPLIFYING AND DETECTING POLYNUCLEOTIDES IN PARALLEL	1. A method of carrying out amplification on a plurality of samples, the method comprising: introducing each of the plurality of samples into an amplification zone of a lane of a multi-lane microfluidic cartridge; isolating each of the plurality of samples within the multi-lane microfluidic cartridge; independently thermally cycling each amplification zone of each lane with a plurality of heaters, each heater of the plurality of heaters associated with each amplification zone being separately controllable, wherein the plurality of heaters cycle between termperatures; and amplifying polynucleotides contained within each of the plurality of samples.	Mar. 2027

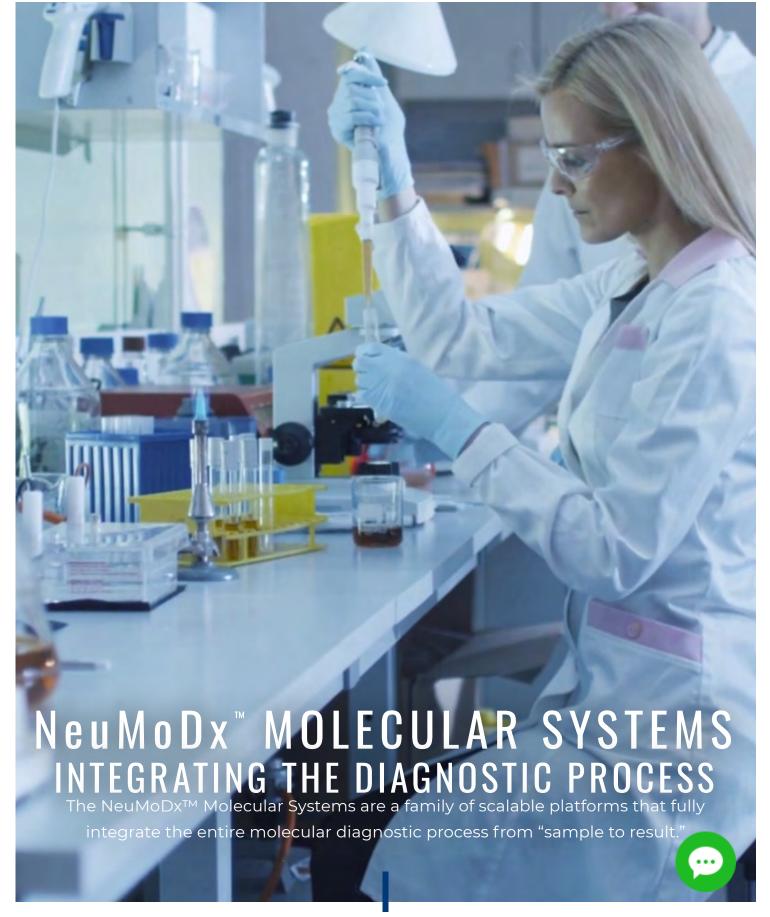
USA	16/158,752	POLYNUCLEOTIDE CAPTURE MATERIALS, AND METHODS OF USING SAME	Claims to be filed in prelim amdnt to be filed in US and also JP:  A method for nucleic acid isolation comprising:  receiving into a process chamber a biological sample containing nucleic acid:  contacting the biological sample with a plurality of binding particles in the process chamber, the binding particles being coated with cationic polyelectrolyte dendrimer which is amide-bonded to the binding particles,  wherein at least a portion of the nucleic acids in the biological sample become reversibly bonded to the binding particles;  washing the binding particles with a solution characterized by a pH less than 10 while retaining the nucleic acid on the binding particles;  releasing a nucleic acid sample from the binding particles by contacting the binding	Mar. 2027 or Jul. 2028
USA	16/200,356	INTEGRATED HEATER AND MAGNETIC SEPARATOR		Mar. 2027 or Nov. 2027
USA	15/619,753	SYSTEMS AND METHODS FOR THERMAL ACTUATION OF MICROFLUIDIC DEVICES	A microfluidic system, comprising:  a first substrate defining a plurality of microfluidic networks, each processing a distinct and separate liquid sample in its network, each of the microfluidic networks comprises a thermally actulated reaction chamber;  a second substratre defining a plurality of heat sources, each heat source being in thermal communication with a respective reaction chamber; and wherein two heat sources corresponding to two thermally actuated reaction chambers belonging to two distinct microfluidic networks are configured to receive the same current or the same voltage.	Mar. 2021
EP	16191773.7	INTERGRATED APPARATUS FOR PERFORMING NUCLEIC ACID EXTRACTION AND DIAGNOSTIC TESTING ON MULTIPLE BIOLOGICAL SAMPLES	An apparatus for amplifying polynucleotides, comprising:     a bay configured to selectively receive a microfluidic cartridge that includes a plurality of sample lanes each containing an independently configured microfluidic network; at least one heat source thermally coupled to and located in the bay, the at least one heat source configured to heat individual sample lanes in a cartridge received in the bay, wherein the at least one heat source compriseds multilple heaters configured to heat distince locations of the cartridge; and a processor configured to control application of heat to the individual sample lanes, separately, in all simultaneously, and in groups simultaneously.	Jul. 2028
EP	18185265.8	METHODS AND SYSTEMS FOR MICROFLUIDIC PROCESSING		Mar. 2022

### EXHIBIT 10















#### DIAGNOSTICS.

### INDUSTRY'S FIRST TRUE CONTINUOUS RANDOM-ACCESS SOLUTION

NeuMoDx<sup>TM</sup> Molecular Systems provide the industry's first true continuous random-access solution and is scalable to meet the needs of the modern clinical laboratory. The ability to load samples and testing consumables on the fly offers up to 8 hours of operator walkaway capability. Room temperature stable reagents and consumables dramatically reduce waste resulting in unmatched flexibility. Liquid handling and transport is achieved through proven robotic technologies. Our proprietary and unitized microfluidic cartridge features independent lanes allowing for simultaneous processing of sample types and varying assays. This unique integration of robotics with advanced microfluidics, reduces operation to three simple steps providing industry leading usability. These capabilities dramatically improve lab productivity and the ability to provide clinicians with critical information in a timely manner.

WHAT WE DO

#### **MOLECULAR SYSTEMS**

NeuMoDx<sup>™</sup> Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from "sample to result." NeuMoDx<sup>™</sup> 288 and NeuMoDx<sup>™</sup> 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry<sup>™</sup> reagent technology, which integrates magnetic particle affinity capture and real time polymerase chain reaction (PCR) chemistry in a multi-sample microfluidic cartridge.









CONTINUOUS RANDOM-ACCESS | FULLY AUTOMATED

### NeuMoDx™ 288 MOLECULAR SYSTEM













### VISIT US AT THE SHOW







Booth #1637

Booth #2859











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- SIGN UP!













# EXHIBIT 11

















The NeuMoDx™ Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from "sample to result". The NeuMoDx™ 288 and the NeuMoDx™ 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry™ reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi-sample microfluidic cartridge. This technology, combined with a platform, uniquely incorporates robotics and microfluidics that result in higher throughput, improved performance and increased efficiency by eliminating the waste associated with technologies that required reconstitution of lyophilized reagents.

NeuMoDx<sup>™</sup> has recently received FDA 510(k) clearance for its NeuMoDx<sup>™</sup> GBS Assay for the detection of Group B Streptococcus (GBS) DNA in antepartum pregnant women and will continue development of tests to detect and monitor sexually transmitted and infectious diseases. Additionally, NeuMoDx<sup>™</sup> offers a broad range of general purpose reagents and consumables for use by laboratorians in developing qualitative and quantitative Laboratory Developed Tests (LDTs) for use with the NeuMoDx<sup>™</sup> Molecular Systems to detect and amplify DNA and RNA targets.

#### **OUR INSTRUMENTS**

NeuMoDx<sup>™</sup> Molecular Systems are a family of scalable platforms that fully integrate the entire molecular diagnostic process from "sample to result." NeuMoDx<sup>™</sup> 288 and NeuMoDx<sup>™</sup> 96 Molecular Systems are fully automated, continuous random-access analyzers that utilize our proprietary NeuDry<sup>™</sup> reagent technology, which integrates magnetic particle affinity capture and real time Polymerase Chain Reaction (PCR) chemistry in a multi sample microfluidic cartridge.







#### NUUNUUA JU MULLUULAN UIOILM

The NeuMoDx<sup>™</sup> 96 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR.

The NeuMoDx<sup>™</sup> 96 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.



### **DETAILS**

**ABOUT THE SYSTEMS** 









#### NeuMoDx<sup>™</sup> 96 TECHNICAL SPECIFICATION SHEET

#### SCIENTIFIC POSTERS

QUANT HCV CVS

NOVEL DRY REAGENT

QUANT HBV CVS

FULLY AUTOMATED LDT

#### QUICK LINKS

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TECHSUPPORT@NEUMODX.COM

NEUMODX<sup>™</sup> 288 MOLECULAR SYSTEM

### NeuMoDx™ 288 MOLECULAR SYSTEM

The NeuMoDx<sup>™</sup> 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx<sup>™</sup> 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.













### **DETAILS**

#### **ABOUT THE SYSTEMS**

VIDEO | NeuMoDx™ TECHNOLOGY

VIDEO | NeuMoDx™ WORKFLOW

#### SPECIFICATION SHEET

NeuMoDx<sup>™</sup> 288 TECHNICAL SPECIFICATION SHEET









NOVEL DRY REAGENT

QUANT HBV CVS

FULLY AUTOMATED LDT

#### QUICK LINKS

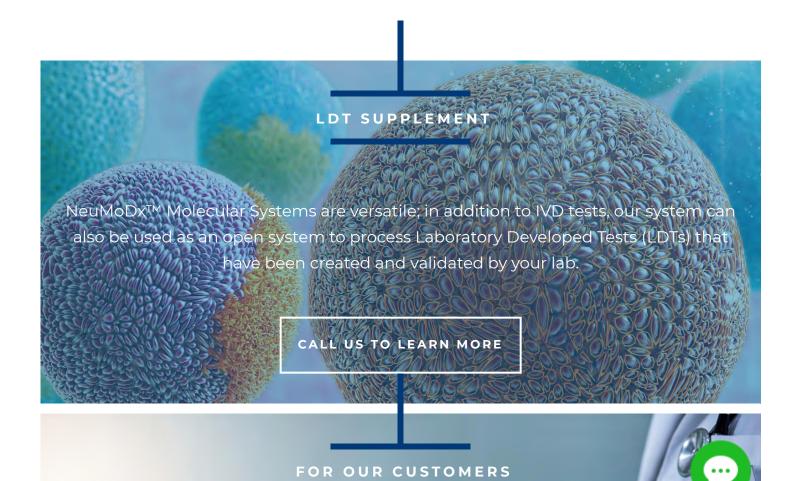
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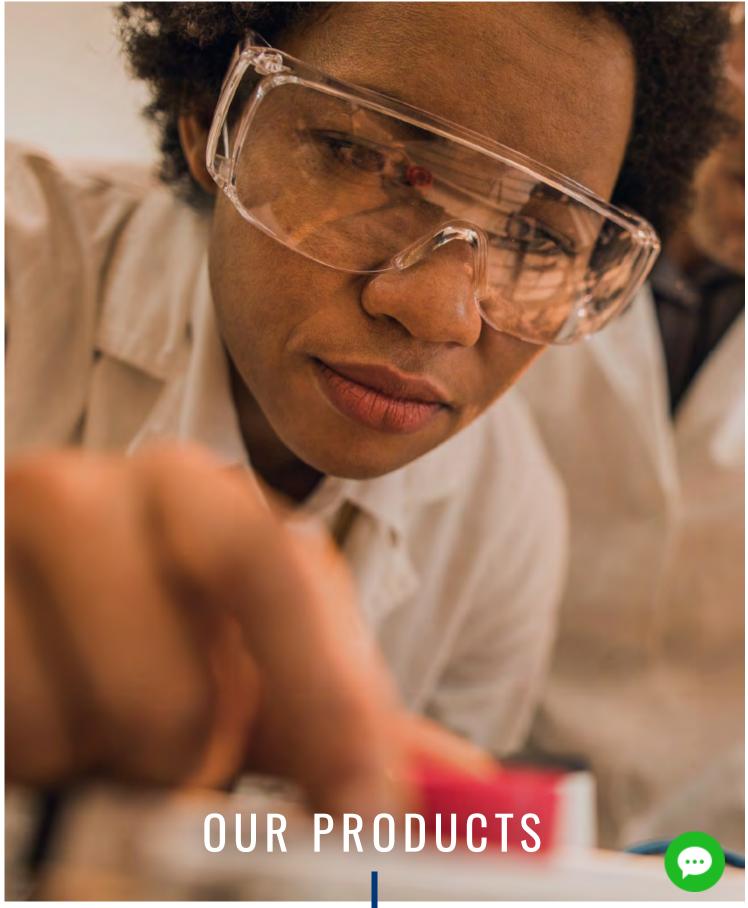


# EXHIBIT 12







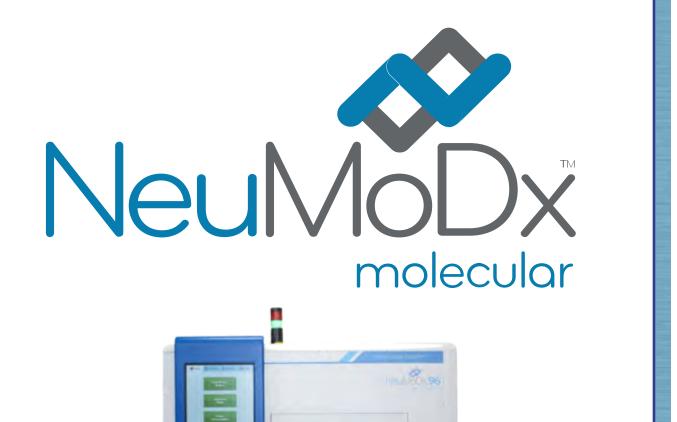












 $\frac{\text{\#500200}}{\text{NeuMoDx}^{\text{TM}}}$  96 Molecular System











# molecular



#500100 NeuMoDx<sup>™</sup> 288 Molecular System

CONSUMABLES

**FDA-CLEARED ASSAYS** 









#200400 NeuMoDx GBS Test Strip

### **CE-IVD ASSAYS**



#200400 NeuMoDx $^{\text{TM}}$  GBS Test Strip

### **LDT TEST STRIPS**













#310100 NeuMoDx LDT Master Mix, RNA



#100400 NeuMoDx LDT Primer/Probe Strip

### **GENERAL USE REAGENTS**











#400100 NeuMoDx<sup>™</sup> Wash Reagent



#400200 NeuMoDx Release Reagent



#100100

 $NeuMoDx^{TM}$  Cartridge











#100200 NeuMoDx<sup>™</sup> Extraction Plate



#235903 CO-RE Tips 300 μL with Filters



#235905

CO-RE Tips 1000  $\mu L$  with Filters











#400400 NeuMoDx Lysis Buffer 1



#400500 NeuMoDx<sup>TM</sup> Lysis Buffer 2



•









#400700 NeuMoDx Lysis Buffer 4

### **NeuMoDx<sup>™</sup> 96 SYSTEM ACCESSORIES**

**NOT PICTURED** 

#400800

NeuMoDx<sup>™</sup> 96 Priming Waste Bottle









### **NOT PICTURED**

#400300

NeuMoDx 288 Priming Waste Bottle



#600800 NeuMoDx<sup>™</sup> 288 Waste Chute

### NeuMoDx<sup>™</sup> 96 AND NeuMoDx<sup>™</sup> 288 SYSTEM ACCESSORIES











NeuMoDx<sup>™</sup> Biohazard Waste Bag



#600200 NeuMoDx Biohazard Waste Container



#600300 NeuMoDx<sup>™</sup> Test Strip Carrier



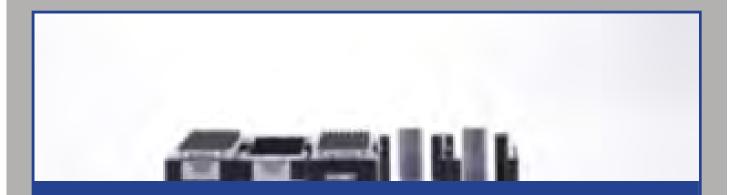






#600400

NeuMoDx<sup>™</sup> Buffer Carrier



#600500

NeuMoDx<sup>™</sup> Tip, Extraction Plate and Filter Carrier



#173410

Specimen Tube Carrier (32 Tube)











#173400

Specimen Tube Carrier (24 Tube)



#173400

NeuMoDx<sup>™</sup> Cartridge Carrier

**NOT PICTURED** 

#600700

NeuMoDx<sup>™</sup> Tip Tray

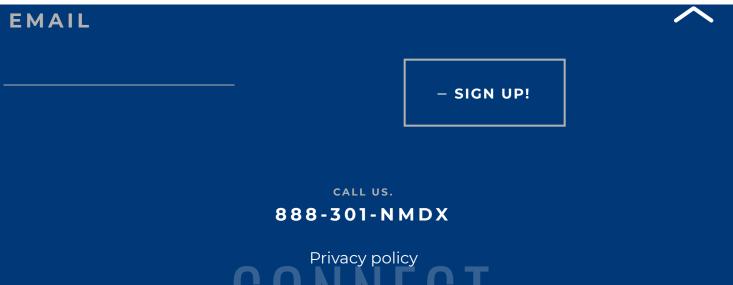














# EXHIBIT 13









CONTINUOUS RANDOM-ACCESS | FULLY AUTOMATED

### NeuMoDx™ 288 MOLECULAR SYSTEM











### **DETAILS**

#### **ABOUT THE SYSTEMS**

VIDEO | NeuMoDx™ TECHNOLOGY

VIDEO | NeuMoDx™ WORKFLOW

#### **SPECIFICATION SHEET**

**(1)** 

NeuMoDx<sup>™</sup> 288 TECHNICAL SPECIFICATION SHEET









QUANT HEVEVS

NOVEL DRY REAGENT
QUANT HBV CVS
FULLY AUTOMATED LDT

#### QUICK LINKS

OVERVIEW

FEATURES AND BENEFITS

#### CONTACT

888-301-NMDX

TECHSUPPORT@NEUMODX.COM

#### ORDER THE PRODUCT

CONTINUOUS RANDOM-ACCESS | FULLY AUTOMATED

### NeuMoDx™ 288 MOLECULAR SYSTEM

#### **OVERVIEW**

NeuMoDx<sup>™</sup> 288 Molecular System is designed for the automated extraction and isolation of nucleic acids, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The NeuMoDx<sup>™</sup> 288 Molecular System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.

#### **Intended Use**

The NeuMoDx<sup>TM</sup> 288 Molecular System is intended for in vitro diagnostic (IVD) use in performing NeuMoDx<sup>TM</sup> validated nucleic acid testing in clinical laboratories. The NeuMoDx<sup>TM</sup> 288 Molecular System is capable of automated extraction and isolation or







quantitative tests, which use NeuMoD $x^{TM}$  -provided consumables and reagents.

#### Instrument Includes:

- Uninterruptible power supply (UPS)
- · Handheld barcode scanner
- · Keyboard and mouse
- NeuMoDx™ Biohazard Waste Container
- Carriers
  - Test Strip Carrier (6)
  - Buffer Carrier (2)
  - · 32-tube Specimen Tube Carrier (9)
  - · Tip, Extraction and Filter Carrier (2)
  - Cartridge Carrier (2)

#### FEATURES AND BENEFITS

- Automatic liquid handling of multiple specimen types, reagents, and buffers
- · Independent lysis heating on 96 locations
- · Independent nucleic acid extraction and purification across 48 locations
- Independent PCR amplification using rapid thermal cycling across all 48 XPCR module locations.
- · Real-time detection of products of amplification
- Fluorescence detection at five wavelengths enabling multiplexed amplification reactions
- · Qualitative or quantitative analysis
- Identification of all consumables and specimen tubes by barcode reader, ensuring complete traceability
- Inventory management to automatically remove empty consumables and replace with new, when applicable
- · Test order verification by NeuMoDx $^{\text{TM}}$  software













### **EMAIL**

- SIGN UP!

CALL US.

888-301-NMDX

Privacy policy

in f



# EXHIBIT 14









CONTINUOUS RANDOM-ACCESS | FULLY AUTOMATED

### NeuMoDx™ 96 MOLECULAR SYSTEM









#### **ABOUT THE SYSTEMS**

VIDEO | NeuMoDx™ TECHNOLOGY

VIDEO | NeuMoDx™ WORKFLOW

#### SPECIFICATION SHEET

NeuMoDx™ 96 TECHNICAL SPECIFICATION SHEET

#### SCIENTIFIC POSTERS

QUANT HCV CVS

NOVEL DRY REAGENT

QUANT HBV CVS

FULLY AUTOMATED LDT

#### QUICK LINKS

OVERVIEW

FEATURES AND BENEFITS

#### CONTACT

888-301-NMDX

TECHSUPPORT@NEUMODX.COM

ORDER THE PRODUCT

CONTINUOUS RANDOM-ACCESS | FULLY AUTOMATED

### NeuMoDx™ 96 MOLECULAR SYSTEM









Haciele acia sequetices by Haciescetice-based FCR. The Neumon

JO MOIECUIAI

System consists of the instrument with touchscreen computer, accessories, and reagents and consumables.

#### Intended Use

The NeuMoDx<sup>™</sup> 96 Molecular System is intended for in vitro diagnostic (IVD) use in performing NeuMoDx<sup>™</sup> validated nucleic acid testing in clinical laboratories. The NeuMoDx<sup>™</sup> 96 Molecular System is capable of automated extraction and isolation of nucleic acids from multiple specimen types, as well as the automated amplification and detection of target nucleic acid sequences by fluorescence-based PCR. The system is capable of providing functionality to enable laboratories to develop qualitative and quantitative tests, which use NeuMoDx<sup>™</sup> provided consumables and reagents.

#### **Instrument Includes:**

- Uninterruptible power supply (UPS)
- Handheld barcode scanner
- Keyboard and mouse
- · Biohazard Waste Bin
- Biohazard Tip Waste Bin
- · Biohazard Waste Container
- Carriers
  - Test Strip Carrier (4)
  - Buffer Carrier (1)
  - 32-tube Specimen Tube Carrier (3)
  - Tip, Extraction and Filter Carrier (1)
  - · Cartridge Carrier (1)

#### FEATURES AND BENEFITS

- · Automatic liquid handling of multiple specimen types, reagents, and buffers
- · Independent lysis heating on 48 locations
- · Independent nucleic acid extraction and purification across 24 locations

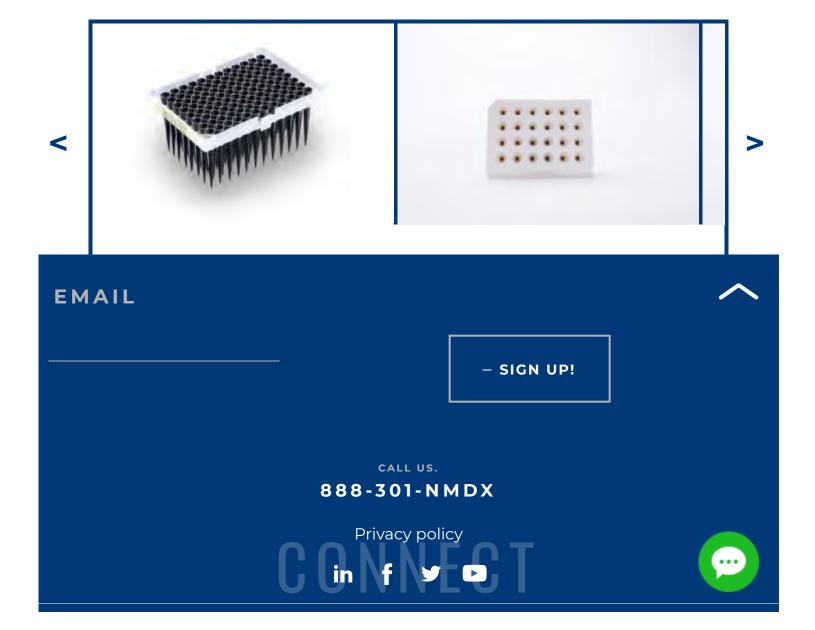








- · Real-time detection of products of amplimeation
- Fluorescence detection at five wavelengths enabling multiplexed amplification reactions
- · Qualitative or quantitative analysis
- Identification of all consumables and specimen tubes by barcode reader, ensuring complete traceability
- Inventory management to automatically remove empty consumables and replace with new, when applicable
- Test order verification by NeuMoDx™ 96 software



# EXHIBIT 15



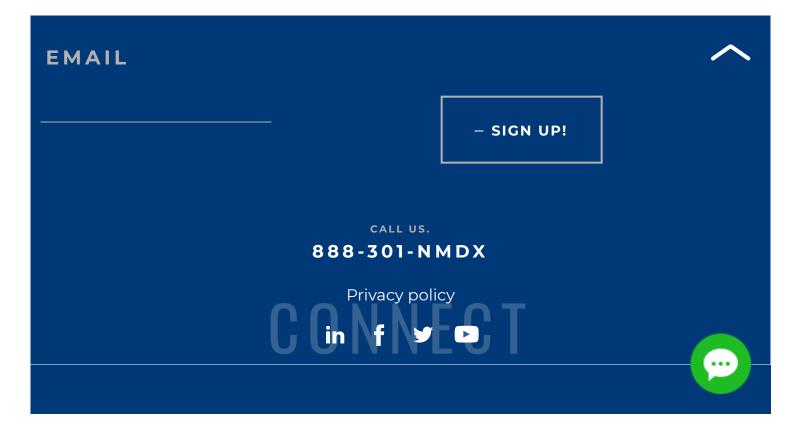




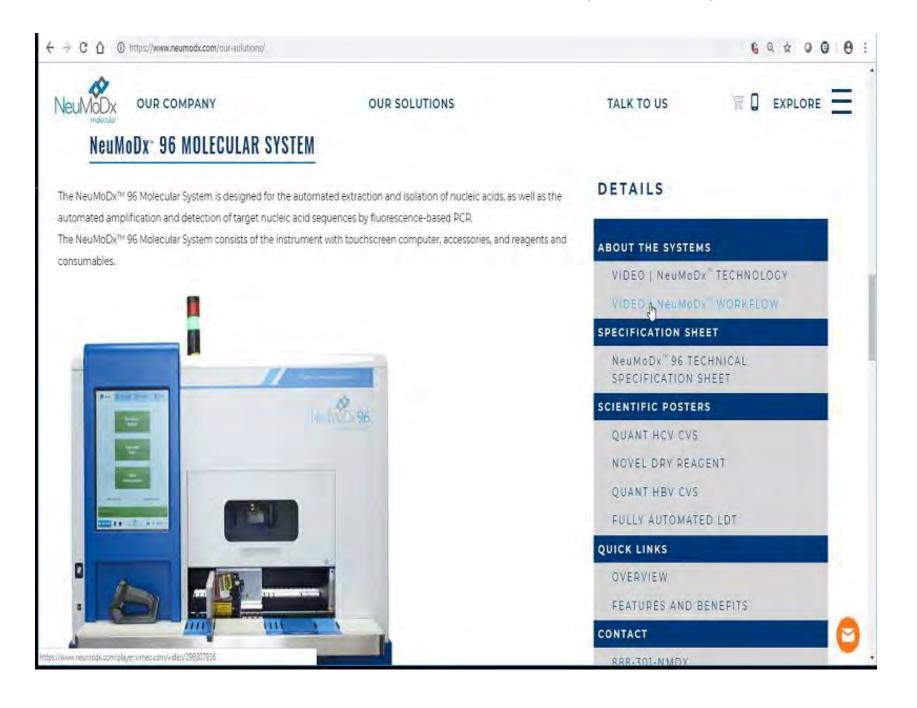
## **PATENTS**

Product	Patents
CARTRIDGE	US Patent Nos. 9,738,887; 9,433,940; 9,101,930; 9,403,165; and 9,452,430. AU Patent No. 2013221701. JP Patent No. 6061313.
P02 (overall system and method)	US Patent Nos. 9,050,594; 9,339,812; 9,441,219; 10,041,062; 9,604,213; and 10,010,888. CN Patent No. ZL 2013 8 00092863.
EXTRACTION PLATE	US Patent Nos. 9,382,532; and 9,540,636.
XPCR MODULE	US Patent Nos. 9,499,896; 9,539,576; 9,637,775; and 10,093,963.

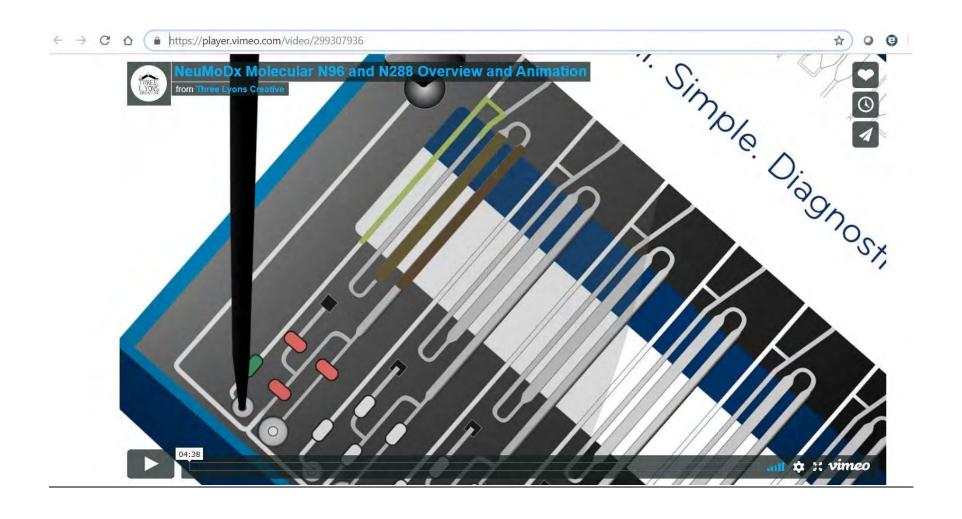
<sup>\*</sup> Other US and foreign patents pending.



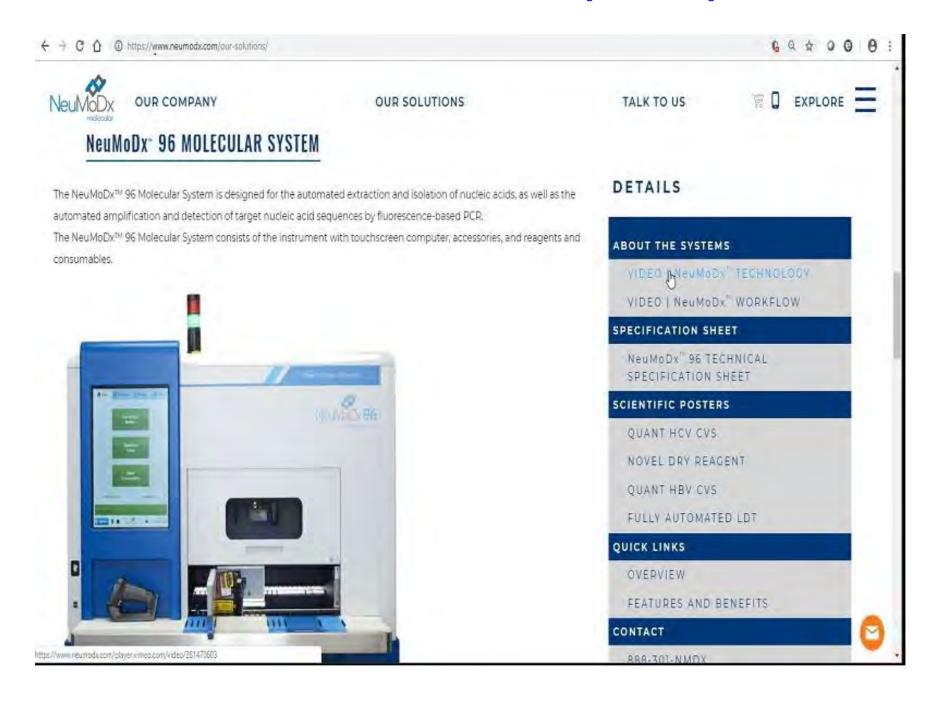
# EXHIBIT 16



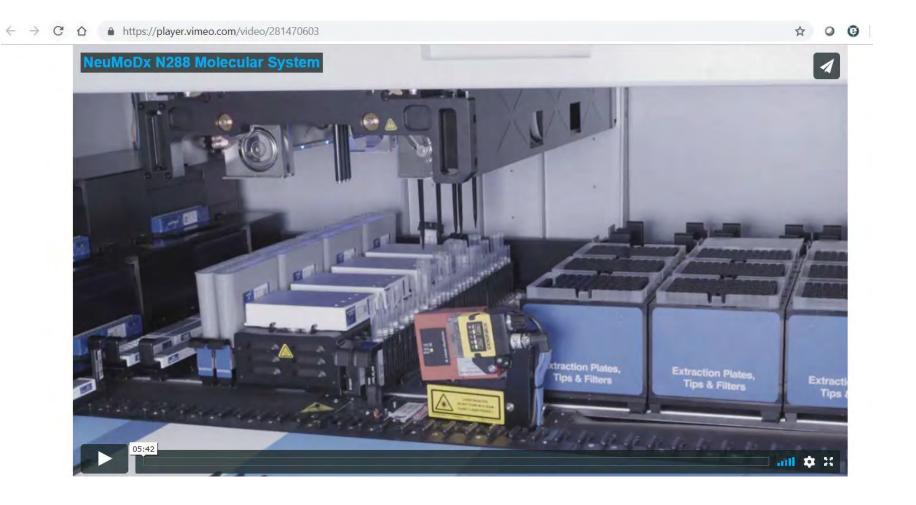
Available at <a href="http://www.neumodx.com/our-solutions/">http://www.neumodx.com/our-solutions/</a> - linking to "VIDEO | NeuMoDx<sup>TM</sup> WORKFLOW" hyperlink at <a href="https://player.vimeo.com/video/299307936">https://player.vimeo.com/video/299307936</a> (last visited June 13, 2019).



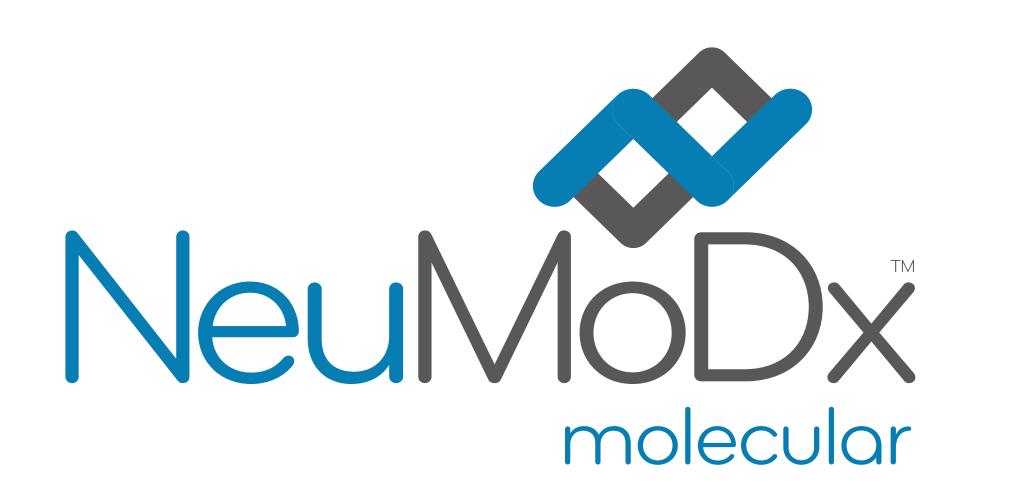
# EXHIBIT 17



Available at <a href="http://www.neumodx.com/our-solutions/">https://www.neumodx.com/our-solutions/</a> - linking to "VIDEO | NeuMoDx<sup>TM</sup> TECHNOLOGY" hyperlink at <a href="https://player.vimeo.com/video/281470603">https://player.vimeo.com/video/281470603</a> (last visited June 13, 2019).



# EXHIBIT 18



# QUANTITATIVE DETECTION OF HCV USING A FULLY INTEGRATED "SAMPLE TO RESULT" MOLECULAR DIAGNOSTIC SYSTEM



Elizabeth Craig\*, Jessica Zhu, Maureen Carey, Catherine Couture, Hui Lin Lee, Michelle Mastronardi, Betty Wu, Sundu Brahmasandra, NeuMoDx Molecular, Ann Arbor, MI

# BACKGROUND

Determining Hepatitis C Virus (HCV) RNA levels in plasma and/or serum is an important tool to characterize viral loads in infected patients to monitor disease progression, efficacy of antiviral therapies, as well as to detect drug resistant mutants and identify relapse upon discontinuation of an antiviral therapy. The NeuMoDx HCV Test is an in-vitro diagnostic assay incorporating a universal nucleic acid isolation chemistry enabling extraction of qPCR ready RNA from serum and plasma specimens, combined with a sensitive quantitative rt-PCR assay to deliver highly accurate results in a completely automated, "random access" manner on the NeuMoDx Molecular System. In addition, all reagents and disposables are room temperature stable and are intended to remain on-board the system to provide a seamless, on-demand testing workflow.

# METHODS

The NeuMoDx Molecular System automates and integrates the extraction, purification, quantification, and results interpretation of infectious disease nucleic acid targets using quantitative RT-PCR. The objective of this study was to test and report performance of the NeuMoDx HCV Test in key analytical performance metrics. Internal pre-analytical studies were performed to characterize the analytical sensitivity, linearity, precision, inclusivity, turnaround time, and results processing accuracy and are reported here.

# RESULTS

The NeuMoDx HCV Test showed a detection limit and lower limit of quantification of 10 IU/mL and demonstrated excellent linearity across a 8 Log dynamic range (R<sup>2</sup>>0.98). Reproducibility and precision across multiple systems, operators, and reagent lots was also demonstrated. The NeuMoDx HCV Test showed equivalent detection performance across all relevant HCV genotypes with a Turnaround time (TAT) of ~75 min. The results processing module incorporated for automated processing of data provides accurate quantitative results, and excellent concordance was demonstrated in a method correlation study conducted between the NeuMoDx HCV Test and a reference test. An R<sup>2</sup> >0.95 and bias of HCV viral load output from the two tests of less than 0.5 Log IU/mL was obtained.

NeuMoDx HCV Test LoD

LoD = 8.6 IU/mL [95% CI (7.0,10.5)]

Log<sub>10</sub>[CONC/mL]

—Curve FIT

Experimental

◆ Calculated LoD

# NeuMoDx Molecular System Streamlined Testing



WORKFLOW

Ease of use: Load specimen(s), walk away and results in 75 min

LOAD
SPECIMENS

INITIATE TESTING WITH TWO ON-SCREEN SELECTIONS

RESULT IN ~75 MINUTES



Large walkaway window of up to 288 samples with room temperature stable onboard reagents.



Patented microfluidic cartridges capable of performing independent sample processing and real-time PCR.

# FEATURES

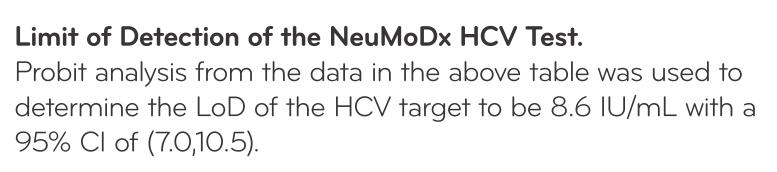
- Integrated Operation: Integrates all steps of molecular diagnostics starting from raw clinical specimens to providing real-time PCR results in a fully automated
- True Random Access: Unlimited ability to mix specimen types and tests
- High Throughput: ~400 DNA tests/~300 RNA tests in an 8 hour shift
- Fast Time to First Results: <75 min for RNA Targets
- Large Walk-Away Window: Up to 288 samples
- Continuous Loading: Specimens and Reagents can be loaded/unloaded at any time
- Seamless On Demand Operation: Automated inventory management of consumables and reagents
- Long In-Use Shelf Life: On-board room temperature stable reagents
- Real-time PCR: Five-color fluorescence detection offers real-time PCR multiplexing ability

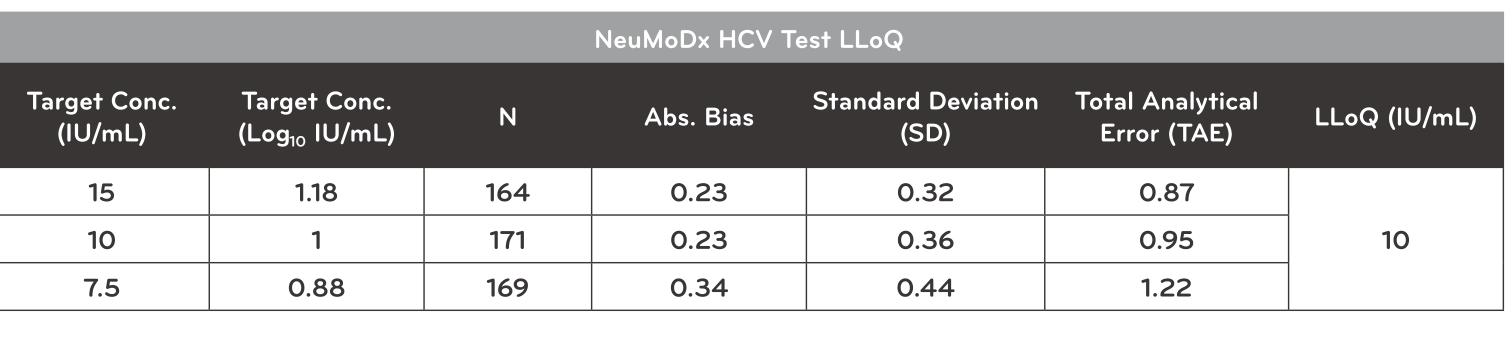
# HCV Analytical Sensitivity (LoD & LLoQ)

The Limit of Detection of the NeuMoDx HCV Test was determined with pooled HCV negative plasma spiked with Acrometrix® HCV Control at three different levels including negative samples. The Limit of Detection of HCV was determined to be 8.6 IU/mL based on Probit style analysis and the calculated LLoQ was determined to be 10 IU/mL.

The NeuMoDx HCV Test further demonstrated sensitivity by accurately detecting all eight HCV genotypes near the limit of detection.

	NeuMoDx HC\	/ Test	Limit of [	Detection	
Target Conc. (IU/mL)	Target Conc. (Log <sub>10</sub> IU/mL)	N	# Positive	% Positive	LoD (Probit) (IU/mL)
100	2	48	48	100%	
15	1.18	118	117	99.1%	
10	1.00	123	121	98.3%	8.6 (7.0,10.5)
7.5	0.88	124	113	91.1%	
0	-	48	0	0%	





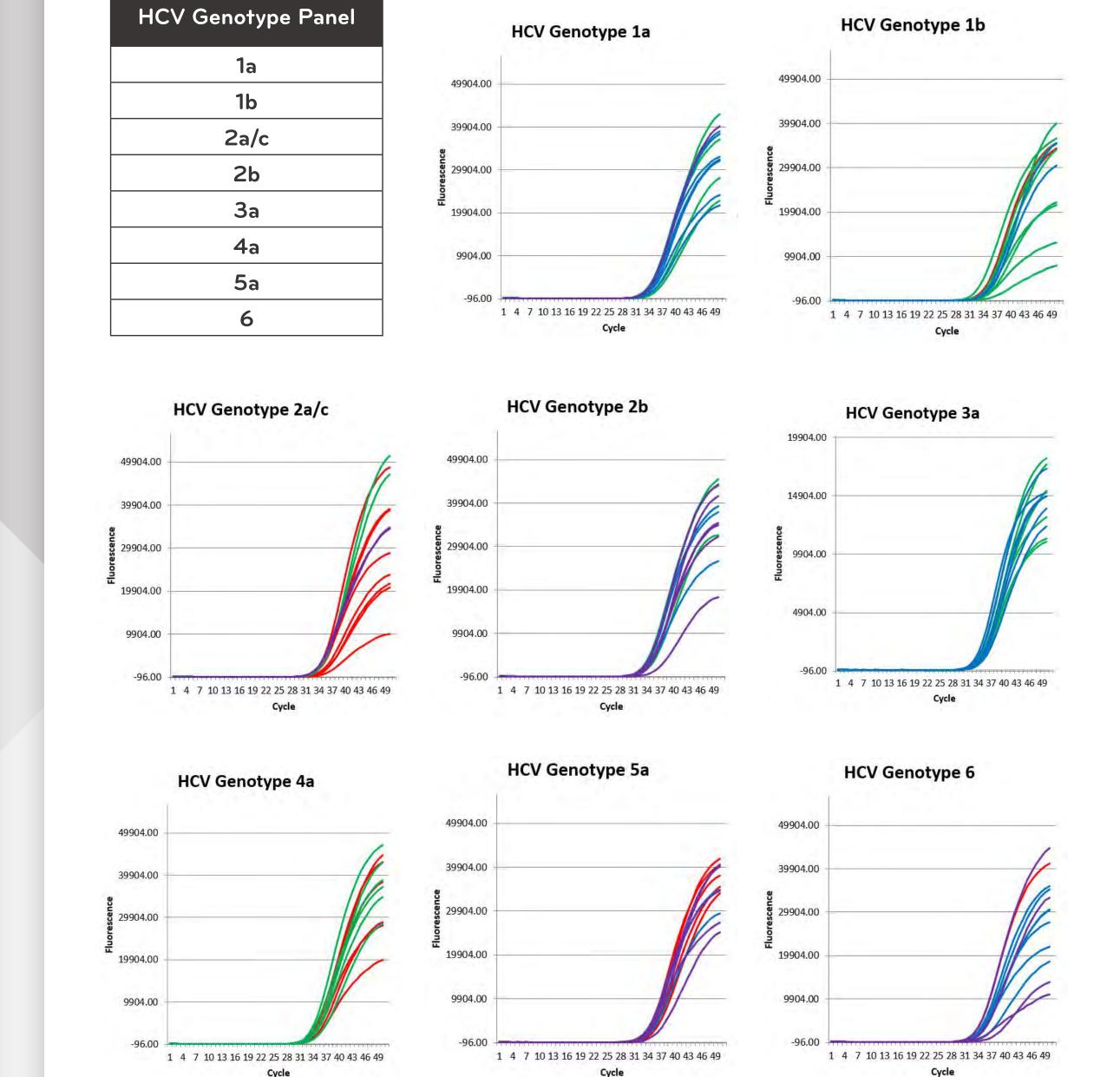
Lower limit of quantitation (LLoQ) of NeuMoDx HCV Test.

The lowest target level detected at a rate > 95% AND with TAE (bias+2\*SD) ≤ 1.0 was used to determine the LLoQ. The LLoQ of the NeuMoDx HCV Test was determined to be ~10 IU/mL.

# Genotype Coverage

HCV genotype inclusivity.

The NeuMoDx HCV Test accurately detected all eight clinically relevant genotypes of HCV (SeraCare HCV RNA Genotype AccuTrak™ Qualification Panel) with 100% amplification near LoD.



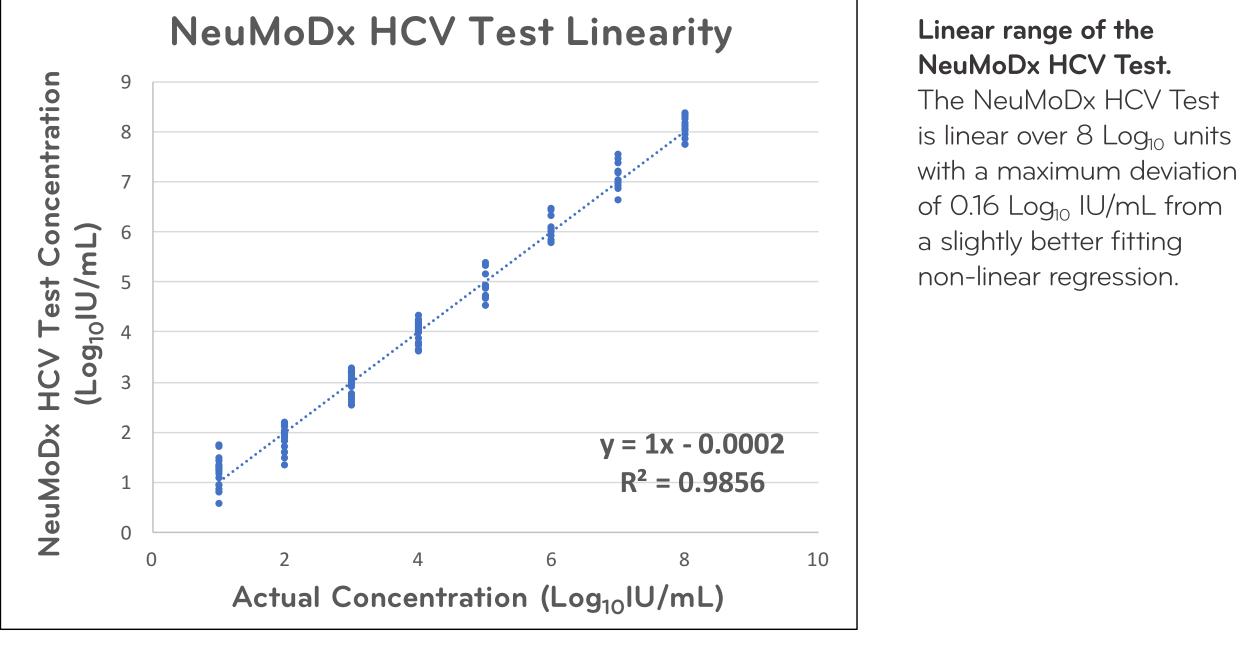
# HCV Linearity

The linearity of the NeuMoDx HCV Test was determined by diluting either Acrometrix HCV RNA or Asuragen HCV Armored RNA® in pooled HCV negative plasma to create a panel spanning eight logs of HCV concentration ranging from 8 Log<sub>10</sub> IU/mL to 1 Log<sub>10</sub> IU/mL.

Data from this study showed that the NeuMoDx HCV Test demonstrated excellent linearity across the 8 logs with a maximum deviation from a slightly better fitting non-linear regression of 0.16 Log<sub>10</sub> IU/mL.

Target Conc. (IU/mL)	Target Conc. (Log <sub>10</sub> IU/mL)	Mean Conc. (Log <sub>10</sub> IU/mL)	Bias	Calculated Linear Fit	Calculated Best Non-Linear Fit	Deviation from Non-Linear Fit
1E+08	8.00	8.05	0.05	8	8.05	0.05
1E+07	7.00	7.10	0.10	7	7.08	0.08
1E+06	6.00	6.03	0.03	6	6.04	0.04
1E+05	5.00	4.87	0.13	5	5.00	0.00
1E+04	4.00	3.96	0.04	4	3.92	-0.08
1E+03	3.00	2.97	0.03	3	2.91	-0.09
1E+02	2.00	1.88	0.12	2	1.97	-0.02
1E+O1	1.00	1.2	0.20	0.99	1.16	0.16
N	NeuMoDx HCV Test Linearity					e of the

NeuMoDx HCV Test Linearity



# Precision

The Within Lab Precision of the NeuMoDx HCV test was determined by testing a 5 member panel of HCV on multiple NeuMoDx Molecular Systems across multiple days. The same concentrations of HCV were used across these consumables and compared to each other for consistency.

The precision Within-run and Across-runs was characterized and the standard deviation for both was determined to be ≤ 0.5 Log<sub>10</sub> IU/mL.

			_			_
Panel Member	Target Conc. (Log <sub>10</sub> IU/mL)		N	Within Run SD	Across Runs SD	Over:
1	5.30	4.95	48	0.23	0.17	0.29
2	4.30	4.03	48	0.23	0.17	0.29
3	3.30	2.99	48	0.11	0.21	0.24
4	2.30	2.05	48	0.12	0.09	0.15
5	1.30	1.05	48	0.50	0.46	0.68

Precision of the NeuMoDx HCV Test.

The NeuMoDx HCV Test demonstrated excellent within laboratory precision calculated from the quantitative data across target levels, reagent lots, and Systems with a maximum overall standard deviation ≤ 0.68 Log<sub>10</sub> IU/mL.

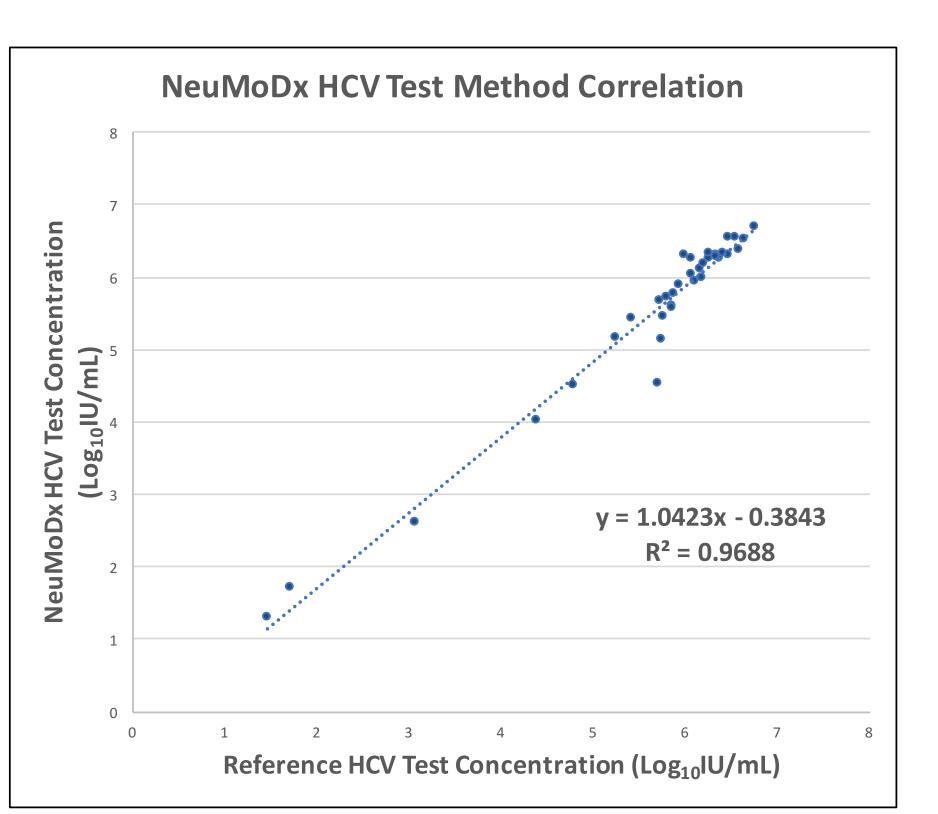
# Method Correlation Study

The quantitative performance of the NeuMoDx HCV Test was assessed against an FDA approved comparator assay by testing clinical specimens from HCV infected patients.

A total of 36 clinical specimens within the linear range were used to generate the linear regression.

Testing was performed internally at NeuMoDx through a single-blinded study using clinical samples obtained from a reference laboratory.

The NeuMoDx HCV test demonstrated excellent quantitative correlation with the reference test.



NeuMoDx HCV Test method correlation.

The NeuMoDx HCV Test demonstrates excellent correlation with the comparator HCV test results from a reference lab.

# CONCLUSION

The NeuMoDx HCV Test is an extremely easy to use, rapid, automated molecular test for the sensitive and accurate viral load monitoring required for effective patient management of HCV infections.

# ACKNOWLEDGMENTS

The authors gratefully acknowledge the help and support provided by all members of the NeuMoDx team. We would also like to thank our collaborators for their invaluable assistance in providing access to clinical specimens for testing and evaluation.

# EXHIBIT 19

#### cv-01126-LPS Document 169-1 Filed 02/25/21 Page 475 of 848 PageID #: 17849

#### **NeuMoDx™** Cartridge INSTRUCTIONS FOR USE



REF

100100 NeuMoDx™ Cartridge

Rx only

|IVD|

For In Vitro Diagnostic Use on the NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems



For detailed instructions, refer to the NeuMoDx™ 288 Molecular System Operator's Manual; p/n 40600108 For detailed instructions, refer to the NeuMoDx™ 96 Molecular System Operator's Manual; p/n 40600317

#### INTENDED USE

The NeuMoDx Cartridge is a proprietary consumable used for the efficacious extraction, purification, amplification and detection of nucleic acids on the NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems (NeuMoDx™ System(s)). The NeuMoDx™ Cartridge is universally used for all tests processed on either NeuMoDx System.

#### **SUMMARY AND EXPLANATION**

Each NeuMoDx Cartridge contains 12 independent microfluidic circuits that enable the independent processing of up to 12 samples once housed appropriately in the XPCR modules of the NeuMoDx System. The NeuMoDx Cartridge also incorporates a chamber to contain all the liquid waste generated in the course of processing the samples.

#### PRINCIPLES OF THE PROCEDURE

The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR. An aliquot of the unprocessed specimen is mixed with the appropriate NeuMoDx™ lysis buffer and subjected to lysis at pre-determined temperatures in the presence of lytic enzymes and magnetic microspheres.

The released nucleic acids are captured by magnetic affinity microspheres and these microspheres (along with the bound nucleic acids) are then loaded into the NeuMoDx Cartridge where the unbound/non-specifically bound components are washed away using the NeuMoDx™ WASH Solution and the bound nucleic acid is eluted using the NeuMoDx™ RELEASE Solution.

The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) as well as the dried Master Mix contained in a NeuMoDx test strip. The system then dispenses the prepared PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.



#### **REAGENTS / CONSUMABLES**

#### **Material Provided**

REF	Contents	Tests per unit	Tests per carton
100100	NeuMoDx™ Cartridge	12	576

#### NeuMoDx™ Reagents and Consumables Required But Not Provided

REF	Contents
400400, 400500 400600, 400700	NeuMoDx™ Lysis Buffer 1, 2, 3 and/or 4
100200	NeuMoDx™ Extraction Plate  Dried magnetic affinity microspheres, lytic enzymes, and sample process controls
400100	NeuMoDx™ WASH Solution
400200	NeuMoDx™ RELEASE Solution
various	NeuMoDx™ test strip (as applicable)
235903	Hamilton CO-RE Tips (300 μL) with Filters (available from NeuMoDx or Hamilton)
235905	Hamilton CO-RE Tips (1000 μL) with Filters (available from NeuMoDx or Hamilton)

#### Other Equipment and Materials Required But Not Provided

NeuMoDx™ 288 Molecular System [REF 500100] OR NeuMoDx™ 96 Molecular System [REF 500200]

#### **NeuMoDx™** Cartridge INSTRUCTIONS FOR USE



REF 100100



#### WARNINGS & PRECAUTIONS

- This consumable is for in vitro diagnostic use with NeuMoDx Systems only.
- Do not use the consumable after the listed expiration date.
- Do not use the consumable if the product or packaging is visibly damaged upon arrival.
- Do not use a NeuMoDx Cartridge that has been dropped; dropping the cartridge can cause invalid results.
- Always handle cartridges by the sides; do not touch the top surface.
- Do not place any labels on the NeuMoDx Cartridge.
- Do not re-use a NeuMoDx Cartridge.
- Do not open a NeuMoDx Cartridge before or after use.
- Always wear clean powder free nitrile gloves when handling specimens or any NeuMoDx reagents or consumables.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in Biosafety in Microbiological and Biomedical Laboratories <sup>1</sup> and in CLSI Document M29-A3 <sup>2</sup>.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.



#### **PRODUCT STORAGE, HANDLING & STABILITY**

- The NeuMoDx Cartridge is stable in the primary packaging at 18 to 28 °C through the stated expiration date on the immediate product label.
- Do not use consumables past the stated expiration date.
- Do not use if the product or packaging has been visually compromised.
- Always handle cartridges by the sides and wear clean powder free, nitrile gloves during any handling.

#### SPECIMEN COLLECTION, TRANSPORT & STORAGE

Validation of optimal Specimen Shipping Conditions and Specimen Stability should be conducted by the user's laboratory for the sample matrix used for each type of test performed.



#### **INSTRUCTIONS FOR USE**

- Open the plastic sleeve and remove a NeuMoDx Cartridge, taking care to only handle the cartridge by the sides and not touching the top surface of the cartridge.
- 2. Touch the arrow below desired Cartridge Carrier icon on the NeuMoDx System touchscreen.
- Place the NeuMoDx Cartridge into the Cartridge Carrier with barcode facing to the right to be read by the barcode scanner; cartridges can be stacked 5X deep in the Cartridge Carrier.
- Touch the arrow again on the touchscreen to load the Carrier into the NeuMoDx System.
- Once the barcode on the NeuMoDx Cartridge is read, the touchscreen will show a green section for cartridges in the loaded carrier. If this does not occur, unload the Carrier and ensure the barcode on the NeuMoDx Cartridge is facing to the right.

#### **LIMITATIONS**

- The NeuMoDx Cartridges can only be used on the NeuMoDx Systems and are not compatible with any other automated molecular diagnostic
- 2. The performance characteristics of lab-developed assays using this consumable must be validated by the user's laboratory before diagnostic claims can be made.
- Because detection of most pathogens is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- 4. Erroneous test results could occur from improper specimen collection, handling, storage, technical error or sample mix-up. In addition, false negative results could occur because the number of organisms in the specimen is below the analytical sensitivity of the test.

NeuMoDx Molecular, Inc. CONFIDENTIAL P/N 40600094 Rev D

### **INSTRUCTIONS FOR USE**



- 5. Use of this consumable is limited to personnel trained on the use of the NeuMoDx System.
- 6. Good laboratory practices, including wearing gloves while loading all reagents and consumables into the system and changing gloves during specimen preparation is critical to reduce chance of contamination.

#### **REFERENCES**

- 1. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. HHS Publication No. (CDC) 21-1112, Revised December 2009
- 2. Clinical And Laboratory Standards Institute (CLSI). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline – Fourth Edition. CLSI document M29-A4; May 2014

#### **TRADEMARKS**

NeuMoDx™ is a trademark of NeuMoDx Molecular, Inc.

TaqMan® is a registered trademark of Roche Molecular Systems, Inc.



### **INSTRUCTIONS FOR USE**



#### **SYMBOLS**

SYMBOL	MEANING
Rx only	Prescription Use Only
<b></b>	Manufacturer
IVD	In Vitro Diagnostic Medical Device
EC REP	EC Representative
REF	Catalog Number
LOT	Batch Code
$\square$	Use By
*	Temperature Limitation
	Humidity Limitation
<b>②</b>	Do Not Reuse
CONTROL	Control
$\overline{\Sigma}$	Contains Sufficient for "n" Tests
Ţ <u>i</u>	Consult Instructions for Use
$\triangle$	Caution
& C €	Biological Risks (Potentially Biohazardous Material)
CE	CE Mark



NeuMoDx Molecular, Inc. 1250 Eisenhower Place Ann Arbor, MI 48108, USA

Contact Number: 1-844-527-0111

Patent: <a href="https://www.neumodx.com/patents">www.neumodx.com/patents</a>



Emergo Europe Prinsessegracht 20 2514 AP The Hauge The Netherlands

**C**€ <sub>0086</sub>

# EXHIBIT 20



#### **NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE**





#### 400200 NeuMoDx™ RELEASE Solution

Rx only



For In Vitro Diagnostic Use on the NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems



For detailed instructions, refer to the NeuMoDx™ 288 Molecular System Operator's Manual; p/n 40600108 For detailed instructions, refer to the NeuMoDx™ 96 Molecular System Operator's Manual; p/n 40600317

#### **INTENDED USE**

The NeuMoDx™ RELEASE Solution is a proprietary reagent used for the efficacious extraction of nucleic acids on a NeuMoDx™ 288 and NeuMoDx™ 96 Molecular Systems (NeuMoDx™ System(s)) in conjunction with other NeuMoDx™ reagents, such as the NeuMoDx™ Extraction Plate, NeuMoDx™ lysis buffers, and NeuMoDx™ WASH Solution.

#### **SUMMARY AND EXPLANATION**

NeuMoDx™ RELEASE Solution is a proprietary reagent that releases captured nucleic acid from NeuMoDx™ proprietary affinity magnetic microspheres providing the eluate at the proper pH for mixing with dried reagents in a NeuMoDx™ test strip and subsequent Real-Time PCR.

#### PRINCIPLES OF THE PROCEDURE

The NeuMoDx Systems use a combination of heat and proprietary extraction reagents to perform cell lysis, nucleic acid extraction and inactivation/reduction of inhibitors from unprocessed clinical specimens prior to presenting the extracted nucleic acid for detection by Real-Time PCR. An aliquot of the unprocessed specimen is mixed with the appropriate NeuMoDx lysis buffer and subjected to lysis at pre-determined temperatures in the presence of lytic enzymes and magnetic microspheres.

The released nucleic acids are captured by magnetic affinity microspheres and these microspheres (along with the bound nucleic acids) are then loaded into the NeuMoDx™ Cartridge where the unbound/non-specifically bound components are washed away using the NeuMoDx WASH Solution and the bound nucleic acid is eluted using NeuMoDx RELEASE Solution.

The NeuMoDx Systems mix the released nucleic acid with assay specific primers and probe(s) and the dried Master Mix contained in a NeuMoDx test strip. The System then dispenses the prepared RT-PCR-ready mixture into the NeuMoDx Cartridge where Real-Time PCR occurs.



#### **REAGENTS / CONSUMABLES**

#### **Material Provided**

REF	Contents	Tests per unit	Tests per carton
400200	NeuMoDx™ RELEASE Solution	~ 1,000*	~ 2,000*

<sup>\*</sup> tests per unit/carton may vary depending on actual use

#### NeuMoDx™ Reagents and Consumables Required But Not Provided

REF	Contents
100200	NeuMoDx™ Extraction Plate  Dried magnetic affinity microspheres, lytic enzymes, and sample process controls
400400, 400500 400600, 400700	NeuMoDx™ Lysis Buffer 1, 2, 3 and/or 4
400100	NeuMoDx™ WASH Solution
100100	NeuMoDx™ Cartridge
various	NeuMoDx™ test strip
235903	Hamilton CO-RE Tips (300 μL) with Filters (available from NeuMoDx or Hamilton)
235905	Hamilton CO-RE Tips (1000 μL) with Filters (available from NeuMoDx or Hamilton)

#### Other Equipment and Materials Required But Not Provided

NeuMoDx™ 288 Molecular System [REF 500100] OR NeuMoDx™ 96 Molecular System [REF 500200]

#### **NeuMoDx™ RELEASE Solution**

#### **INSTRUCTIONS FOR USE**



400200



#### **WARNINGS & PRECAUTIONS**

- This reagent is for in vitro diagnostic use with NeuMoDx Systems only.
- Do not use the reagents after the listed expiration date.
- Do not use if the safety seal is broken, if the packaging is damaged upon arrival, or if signs of leakage are present.
- Ensure that NeuMoDx RELEASE Solution is at room temperature before use the NeuMoDx System.
- Do not reuse any NeuMoDx consumable or reagent.
- NeuMoDx RELEASE Solution is high in pH and should be handled with care, refer to SDS for more specific information.
- Safety Data Sheets (SDS) are provided for each reagent.
- Always wear clean powder free nitrile gloves when handling specimens or any NeuMoDx reagents and consumables.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in Biosafety in Microbiological and Biomedical Laboratories 1 and in CLSI Document M29-A3 2.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.



#### **PRODUCT STORAGE, HANDLING & STABILITY**

- NeuMoDx RELEASE Solution is stable in the primary packaging at 18 to 28 °C through the stated expiration date on the immediate product label.
- Do not use reagents past the stated expiration date.
- Do not use if the primary or packaging has been visually compromised.
- NeuMoDx RELEASE Solution placed in the Reagent drawer of the NeuMoDx System is stable for 1 month when operating within the environmental conditions specified in the NeuMoDx™ 288/96 Molecular System Operator's Manual(s). The NeuMoDx System software will prompt the removal of NeuMoDx RELEASE Solution that has been in-use for longer than 1 month.

#### **SPECIMEN COLLECTION, TRANSPORT & STORAGE**

Validation of optimal Specimen Shipping Conditions and Specimen Stability should be conducted by the user's laboratory for the sample matrix used for each type of test performed.



#### **INSTRUCTIONS FOR USE**

- 1. The NeuMoDx System will be pre-loaded with NeuMoDx RELEASE Solution when installed and qualified.
- 2. To change the NeuMoDx RELEASE Solution, touch the arrow below the release solution icon on the NeuMoDx System touchscreen to unlock the appropriate Bulk Reagent Drawer (A or B) and follow the on-screen instructions.
  - a. Open Bulk Reagent Drawer (A or B).
  - b. Use the handheld barcode scanner to scan the barcode of the new NeuMoDx RELEASE Solution.
  - c. Remove and discard the temporary cap from the new NeuMoDx RELEASE Solution.
  - d. Without setting the tubing on any surface to avoid the risk of contamination, disconnect the cap with affixed black tubing from the current NeuMoDx RELEASE Solution.
  - e. Immediately place cap with affixed tubing into the new NeuMoDx RELEASE Solution. Turn cap to tighten.
  - f. Consult product SDS for proper disposal.

### NeuMoDx

### NeuMoDx™ RELEASE Solution INSTRUCTIONS FOR USE



400200

#### **LIMITATIONS**

- NeuMoDx RELEASE Solution can only be used on the NeuMoDx System and is not compatible with any other automated molecular diagnostic system.
- The performance characteristics of user assays using this reagent is unknown and must be validated by your laboratory before diagnostic claims can be made.
- 3. Care must be taken when changing NeuMoDx RELEASE Solution in the Reagent Drawer to not contaminate the tubing and to avoid prolonged exposure to air.
- 4. Because detection of most pathogens is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
- 5. Erroneous test results could occur from improper specimen collection, handling, storage, technical error or sample mix-up. In addition, false negative results could occur because the number of organisms in the specimen is below the analytical sensitivity of the test.
- 6. Use of this reagent is limited to personnel trained on the use of the NeuMoDx System.
- Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens.

#### **REFERENCES**

- Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5<sup>th</sup> edition. HHS Publication No. (CDC) 21-1112, Revised December 2009
- 2. Clinical And Laboratory Standards Institute (CLSI). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline Fourth Edition. CLSI document M29-A4; May 2014

#### **TRADEMARKS**

NeuMoDx™ is a trademark of NeuMoDx Molecular, Inc.

TaqMan® is a registered trademark of Roche Molecular Systems, Inc.

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P/N 40600101 Rev D

### **INSTRUCTIONS FOR USE**



#### **SYMBOLS**

SYMBOL MEANING			
Rx only	MEANING  Prescription Use Only		
IX Only	Trescription osc only		
•••	Manufacturer		
IVD	<i>In Vitro</i> Diagnostic Medical Device		
EC REP	EC Representative		
REF	Catalog Number		
LOT	Batch Code		
Σ	Use By		
1	Temperature Limitation		
	Humidity Limitation		
<b>\oint\oint\oint\oint\oint\oint\oint\oint</b>	Do Not Reuse		
CONTROL	Control		
Σ	Contains Sufficient for "n" Tests		
Ţi	Consult Instructions for Use		
<u> </u>	Caution		
<u></u>	Biological Risks (Potentially Biohazardous Material)		
<b>&amp; C €</b>	CE Mark		



NeuMoDx Molecular, Inc. 1250 Eisenhower Place Ann Arbor, MI 48108, USA

Contact Number: 1-844-527-0111

Patent: <a href="https://www.neumodx.com/patents">www.neumodx.com/patents</a>



Emergo Europe Prinsessegracht 20 2514 AP The Hauge The Netherlands

 $\epsilon$ 

# EXHIBIT 21

#### NeuMoDx<sup>™</sup> 96 Molecular System







#### **Performance Specifications**

Time to first result	~ 60 minutes (DNA) / ~ 80 minutes (RNA)		
Tests/hour	Up to 18/hour¹		
Maximum throughput	Up to 144/8 hours <sup>2</sup>		
Walk-away time	6 hours		
Maximum number of tests per run	Continuous, Random-Access		
Sample capacity	96 initial load; Continuous, Random-Access Thereafter		
Reagent capacity	320 initial load; Continuous, Random-Access Thereafter		
NeuDry™ Test Strip stability	Onboard Stability: 10 days³ Room Temperature Stability: 540 days Multi-well extraction plate may remain onboard until fully utilized, up to 10 days		
Results released	Results released continuously after first resul		
3-Step operation	<ol> <li>Place specimens, test strips and consumables onto carriers</li> <li>Place carriers on autoloader shelf</li> <li>Press "Load"</li> </ol>		
Operational flexibilit	Continuous Random-Access Perform LDT Qualitative and Quantitative assays simultaneously on demand <sup>4</sup> Onboard inventory management Simultaneous use of multiple tube types and sizes Flexible specimen tube compatibility • Diameter: 11 mm - 18 mm • Height: 60 mm - 120 mm		
Barcode symbology	Code 128 Code 39 Code 39 ASCII		

Future Software upgrades will increase the throughput to ~21/hour. \*Future software upgrades will increase this throughput to ~175/8 hours. \*Future testing anticipated to demonstrate >28 days stability. \*Future release planned for IVD menu.

Electrical Specifications	230 V	120 V
Maximum power input	1,920 W	1,920 W
Input voltage	230 VAC+/- 10%	120 VAC+/- 10%
Max current	8 A	16 A
Frequency	50 Hz	60 Hz
Installation category	II	II
Pollution degree	2	2
Degree of protection	IP 00	IP 00

Optical Wavelengths	Excitation (nm)	Emission (nm)
1	470	510
2	530	555
3	585	610
4	625	660
5	680	715 long pass

#### **Physical Dimensions**

Width	137.2 cm (54 in.)
Depth	109.2 cm (43 in.)
Height	109.2 cm (43 in.)
Total weight	272 kg (600 lb)

#### **Environmental Requirements**

Operating temperature	18–28°C
Ambient operating humidity	20-60% non-condensing
Maximum altitude	2,000 m (6,562 ft)
Non-operating temperature	-20–60°C
Non-operating relative humidity	20-90% non-condensing
Noise level	<65 dBA
Operation location	For indoor use only

### EXHIBIT 22

### NeuMoDx<sup>™</sup> 288 Molecular System







#### **Performance Specifications**

Time to first result	~ 60 minutes (DNA) / ~ 80 minutes (RNA)
Tests/hour	Up to 36/hour¹
Maximum throughput	Up to 288/8 hours²
Walk-away time	8 hours
Maximum number of tests per run	Continuous, Random-Access
Sample capacity	288 initial load; Continuous, Random-Access Thereafter
Reagent capacity	480 initial load; Continuous, Random-Access Thereafter
NeuDry™ Test Strip stability	Onboard Stability: 28 days Room Temperature Stability: 540 days Multi-well extraction plate may remain onboard until fully utilized, up to 10 days
Results released	Results released continuously after first resul
3-Step operation	Place specimens, test strips and consumables onto carriers     Place carriers on autoloader shelf     Press "Load"
Operational flexibilit	Continuous Random-Access Perform IVD and LDT Qualitative and Quantitative assays simultaneously on demand Onboard inventory management Simultaneous use of multiple tube types and sizes Flexible specimen tube compatibility  • Diameter: 11 mm - 18 mm  • Height: 60 mm – 120 mm
Barcode symbology	Code 128 Code 39 Code 39 ASCII

 $^1$ Future software upgrades will increase this throughput to  $\sim\!\!42/\!$ hour.  $^2$ Future software upgrades will increase this throughput to  $\sim\!\!350/8$  hours.

Electrical Specifications	230 V	120 V
Maximum power input	1,920 W	1,920 W
Input voltage	230 VAC+/- 10%	120 VAC+/- 10%
Max current	8 A	16 A
Frequency	50 Hz	60 Hz
Installation category	II	II .
Pollution degree	2	2
Degree of protection	IP 00	IP 00

Optical Wavelengths	Excitation (nm)	Emission (nm)
1	470	510
2	530	555
3	585	610
4	625	660
5	680	715 long pass

#### **Physical Dimensions**

Width	182.9 cm (72 in.)	
Depth	109.2 cm (43 in.)	
Height	190.5 cm (75 in.)	
Total weight	>408 kg (>900 lb)	

#### **Environmental Requirements**

Operating temperature	18–28°C
Ambient operating humidity	20-60% non-condensing
Maximum altitude	2,000 m (6,562 ft)
Non-operating temperature	-20-60°C
Non-operating relative humidity	20-90% non-condensing
Noise level	<65 dBA
Operation location	For indoor use only

# EXHIBIT 23



June 26, 2018

NeuMoDx Molecular, Inc. % Kay Fuller Principal Consultant and Official Correspondent Medical Device Regulatory Solutions, LLC 230 Collingwood Dr. Suite 260 Ann Arbor, Michigan 48103

Re: K173725

Trade/Device Name: NeuMoDx GBS Assay Regulation Number: 21 CFR 866.3740

Regulation Name: Streptococcus spp. serological reagents

Regulatory Class: Class I Product Code: NJR, OOI Dated: March 23, 2018 Received: March 29, 2018

#### Dear Kay Fuller:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the <u>Federal Register</u>.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

Page 2 - Kay Fuller K173725

803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <a href="http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm">http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm</a>.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (<a href="https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/">https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/</a>) and CDRH Learn (<a href="http://www.fda.gov/Training/CDRHLearn">http://www.fda.gov/Training/CDRHLearn</a>). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (<a href="http://www.fda.gov/DICE">http://www.fda.gov/DICE</a>) for more information or contact DICE by email (<a href="DICE@fda.hhs.gov">DICE@fda.hhs.gov</a>) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar - S<sub>For</sub>

Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

#### Case 1:19-cv-01126-LPS | Document 169-1 | Filed 02/25/21 | Page 493 of 848 PageID #: 17867

DEPARTMENT OF HEALTH AND HUMAN SERVICES Food and Drug Administration

#### Indications for Use

510(k) Number (if known)

K173725

Form Approved: OMB No. 0910-0120

Expiration Date: 06/30/2020 See PRA Statement below.

CONTINUE ON A SEPARATE PAGE IF NEEDED.	_
Prescription Use (Part 21 CFR 801 Subpart D) Over-The-Counter Use (21 CFR 801 Subpart C)	
Type of Use (Select one or both, as applicable)	_
The NeuMoDx <sup>TM</sup> GBS Assay does not provide susceptibility results. Cultured isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.	
Indications for Use (Describe) The NeuMoDx <sup>TM</sup> GBS Assay as implemented on the NeuMoDx <sup>TM</sup> 288 Molecular System is a qualitative in vitro diagnostic test designed to detect Group B Streptococcus (GBS) DNA from 18-24 hour Lim broth enrichments of vaginal/rectal swabs from pregnant women. The test incorporates automated DNA extraction to isolate the target nucleic acid from the specimen and real-time polymerase chain reaction (PCR) to detect an 88 bp region of the pcsB gene sequence in the Streptococcus agalactiae chromosome. Results from the NeuMoDx <sup>TM</sup> GBS Assay can be used as an aid in determining colonization status in antepartum women.	
Device Name NeuMoDx™ GBS Assay	

This section applies only to requirements of the Paperwork Reduction Act of 1995.

#### \*DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.\*

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

> Department of Health and Human Services Food and Drug Administration Office of Chief Information Officer Paperwork Reduction Act (PRA) Staff PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

#### 14. 510(k) SUMMARY

#### NeuMoDx Molecular, Inc.

#### NeuMoDx™ GBS Assay

June 25, 2018

#### 1. GENERAL INFORMATION

**Submitter Information:** NeuMoDx Molecular, Inc.

1250 Eisenhower Place Ann Arbor, MI 48108 USA

**Contact Information:** 

**Primary Contact:** Kay Fuller, RAC

Principal Regulatory Consultant

Medical Device Regulatory Solutions, LLC

734-846-7852

Secondary Contact: Dawn Ross

Sr. Director Quality Assurance NeuMoDx Molecular, Inc.

#### 2. DEVICE INFORMATION

**Device Name:** GBS Assay

**Proprietary Name:** NeuMoDx™ GBS Assay

Common Name: Group B Strep Assay

Classification Name: Nucleic Acid Amplification Assay System, Group B

Streptococcus, Direct Specimen Test

Classification Code: NJR - Primary

OOI - Secondary

Classification: Class I

FDA Review Panel: 83 - Microbiology

**Regulation Number:** 21 CFR §866.3740

**3. PREDICATE DEVICE** BD Max<sup>™</sup> GBS Assay (K090191)

#### 4. DEVICE DESCRIPTION

The NeuMoDx<sup>™</sup> GBS Assay (Subject Device) as implemented on the NeuMoDx<sup>™</sup> 288 Molecular System is an automated, qualitative, in vitro diagnostic test for the detection of group B *Streptococcus* (GBS), also

known as *Streptococcus agalactiae* from vaginal/rectal swabs collected from pregnant women at 35 - 37 weeks of gestation and enriched in a commercially available Lim broth medium. An aliquot of an overnight Lim broth culture added to the NeuMoDx™ GBS Assay is used for the testing. All further specimen handling is automated.

The GBS Assay test strip, in combination with required NeuMoDx buffers, extraction reagents, wash and release solutions, as well as the microfluidic cartridge (non-active,) and the fully automated NeuMoDx™ 288 Molecular System (a real time nucleic acid amplification system), utilizes real-time polymerase chain reaction (PCR) for the amplification of GBS DNA and fluorogenic target-specific TaqMan® probes for the detection of the amplified GBS DNA. General use components and the System are packaged and provided separately by NeuMoDx.

After the test is processed, a determination of the presence/absence of GBS DNA in the specimen is automatically made based on the amplification status of GBS and the Sample Process Control using preestablished decision criteria. The test results will be reported as Negative, Positive, Indeterminate or Unresolved based on the amplification status of the target and sample processing control. Results are reported based on the decision algorithm noted in Table 1.

Table 1

Result	Result GBS C <sub>t</sub> Sample Proce		
Positive	9 < <b>C</b> <sub>t</sub> < 37 And EP > 3000	N/A	
Negative	N/A <b>OR</b> C <sub>t</sub> < 9 <b>OR</b> > 37	25 < C <sub>t</sub> < 35 And EP > 2000	
Indeterminate	N/A SYSTEM ERROR NOTED	N/A SYSTEM ERROR NOTED	
Unresolved Not detected Not detected		Not detected	

EP = End Point Fluorescence (after baseline correction)

External controls are not provided by NeuMoDx but are recommended to be performed as required by the laboratory's internal procedures.

#### Standards/Guidance Documents Referenced

CLSI Guideline EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline.

#### Test Principle - Summary

The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) using fluorogenic oligonucleotide probe molecules specific to the amplicons for their respective targets.

TaqMan® probes consist of a fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. While the probe

is intact, the fluorophore and the quencher are in proximity, resulting in the quencher molecule quenching the fluorescence emitted by the fluorophore via FRET (Förster Resonance Energy Transfer).

TaqMan® probes are designed such that they anneal within a DNA region amplified by a specific set of primers. As the Taq DNA polymerase extends

the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq DNA polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing detecting fluorescence of the fluorophore. The resulting fluorescence signal detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target DNA present in PCR.

A TaqMan® probe labeled with a fluorophore (Excitation: 490 nm & Emission: 521 nm) at the 5' end, and a dark quencher at the 3' end, is used to detect GBS DNA. For detection of the Sample Process Control, the TaqMan® probe is labeled with an alternate fluorescent dye (Excitation: 535 nm & Emission: 556 nm) at the 5' end, and a dark quencher at the 3' end. The NeuMoDx™ 288 Molecular System monitors the fluorescent signal emitted by the TaqMan® probes at the end of each amplification cycle and presents the test result.

#### 5. INDICATIONS FOR USE

The NeuMoDx™ GBS Assay as implemented on the NeuMoDx™ 288 Molecular System is a qualitative in vitro diagnostic test designed to detect Group B *Streptococcus* (GBS) DNA from 18-24 hour Lim broth enrichments of vaginal/rectal swabs from pregnant women. The test incorporates automated DNA extraction to isolate the target nucleic acid from the specimen and real-time polymerase chain reaction (PCR) to detect an 88 bp region of the *pcsB* gene sequence in the *Streptococcus agalactiae* chromosome. Results from the NeuMoDx™ GBS Assay can be used as an aid in determining colonization status in antepartum women.

The NeuMoDx™ GBS Assay does not provide susceptibility results. Cultured isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women.

### 6. COMPARISON OF TECHNOLOGICAL CHARACTERISTICS

The NeuMoDx<sup>™</sup> GBS Assay's fundamental technological characteristics are similar to those of the predicate device. The NeuMoDx<sup>™</sup> GBS Assay (subject device) is substantially equivalent to the BD Max<sup>™</sup> GBS Assay device (predicate device), noted herein. Both the subject device and predicate device assays detect Group B *Streptococcus* (GBS) DNA from enriched vaginal/rectal swab specimens. Both subject and predicate

assays determine the presence of the target organisms through real-time PCR amplification and fluorogenic target-specific hybridization detection and utilize a similar instrumentation format.

#### **Substantial Equivalence Summary**

		· · · · · · · · · · · · · · · · · · ·	
Feature Comparison Criteria	Subject Device NeuMoDx™ GBS Assay K173725	Predicate Device BD MAX™ GBS Assay K090191	Subject Device SE to K090191?
21 CFR Reg #, Product Code & Classification	21 CFR §866.3740 NJR Class I	21 CFR §866.3740 NJR Class I	Yes
Regulation Name	Nucleic Acid Amplification Assay System, Group B Streptococcus, Direct Specimen Test	Nucleic Acid Amplification Assay System, Group B Streptococcus, Direct Specimen Test	Yes
Prescription Device - Rx Only	Yes	Yes	Yes
Indications for Use	The NeuMoDx™ GBS. Assay as implemented on the NeuMoDx™ 288 Molecular System is a qualitative in vitro disgnostic test designed to detect Group B Streptococcus (GBS) DNA from 19-24 hour Lim broth enrichments of vaginalirectal swabs from pregnant women. The test incorporates automated DNA extraction to isolate the target nucleic acid from the specimen and real-time polymerase chain reaction (PCR) to detect an 88 bp region of the PcBB gene sequence in the Streptococcus agalactiae chromosome. Results from the NeuMoDx™ GBS Assay can be used as an aid in determining colonization status in antepartum women. The NeuMoDx™ GBS Assay does not provide susceptibility testing as recommended for periodilifin-allergic women.	The BD MAX**GBS.Assay as implemented on the BD MAX**System is a qualitative in vitro diagnostic test designed to detect Group B Streptoscocus (GBS) DMA in Lim Broth cultures after incubation for greater than or equal to (>)18 hours, obtained from vaginal and rectal swab specimens from antepartum pregnant women. The test incorporates automated DfA extraction to isolate the farget nucleic add from the specimen and real-time polymerase chain reaction (PCR) to detect a 124 by region of the ctb gene sequence of the Streptococcus agalactae chromosome. Results from the BD MAX**GBS Assay can be used as an aid in determining colonization status in antepartum women.  The BD MAX**GBS Assay does not provide susceptibility results Cultured isolates are needed for performing susceptibility testing as recommended for penicillin altergic women. Subculture to solidmedia for additional testing when indicated.	Yes
Analyte	Group B Streptococcus DNA	Group B Streptococcus DNA	Yes
Specimen Type	Vaginal-rectal swab (Enriched Lim broth 18-24 hrs)	Vaginal-rectal swab (Enriched Lim broth ≥ 18 hrs)	Yes
Specimen Collection Media Type	Amies or Stuart	Amies or Stuart	Yes
Sample Preparation Method	Sample Preparation for Nucleic Acid Extraction is automated on NeuMoDx™ 288 Molecular System	Sample Preparation for Nucleic Acid Extraction is automated on BD MAX System	Yes
Sample Matrix	Enriched in overnight LIM	Enriched in overnight LIM	Yes
Test Reference Comparison Method	CDC GBS 2010 Guidelines of Culture Processing/Identification Procedure	CDC GBS 2002 Guidelines of Culture Processing/Identification Procedure	Yes
Platform	NeuMoDx™ 288 Molecular System (random access)	BD MAX System (random access)	Yes
Assay Format	Amplification: Real Time PCR Detection: Fluorogenic	Real Time Fluorogenic Detection of PCR amplification	Yes
DNA Target Sequence	88 bp region of the PcsB gene sequence in the Streptococcus agalactiae chromosome	124 bp region of the cfb gene sequence of the Streptococcus agalactiae chromosome	Yes
Probes	TaqMan®	Scorpion	Yes
Single Use	Yes	Yes	Yes
User / skill required	Moderate Complexity  Rx only - Qualified Laboratory Personnel Built in protocol No data interpretation required	Moderate Complexity  No special skills required Built in protocol No data interpretation required	Yes
Automatic Assay	Yes - Built-in Result Interpretation	Yes - Built-in Result Interpretation	Yes
Internal Process Control	Sample process control is extracted and amplified with each sample as a process monitor	Extraction and PCR internal process control is a process monitor	Yes
External Control	Not provided by NeuMoDx; commercial materials available. Not required to perform testing. Appropriate controls and testing intervals must be determined by the laboratory	Materials available commercially but not required to run the test	Yes

#### 7. NON-CLINICAL TESTING SUMMARY

#### **Analytical Performance**

#### a. Precision/Reproducibility

#### Precision

Qualitative testing was performed on the NeuMoDx<sup>™</sup> 288 Molecular System using the NeuMoDx<sup>™</sup> GBS Test Strip where 2 runs per day were performed across 3 systems over a period of 12 non-consecutive days. This within-lab precision testing included 2 reagent lots and was performed by 2 operators.

A run was defined as three replicates tested for each of the five different levels shown in Table 2 (True Negative, Low Negative, Moderate Negative, Low Positive and Moderate Positive) for a total of 15 specimens per run per system. Specimens were prepared by spiking cultured GBS into pooled, screened negative clinical remnant Lim broth. For each run performed, a positive and a negative external control were processed in addition to the 15 specimens. A total of 72 runs and 1224 tests were performed in this study, including the external controls. Table 3 shows comparison across instruments. Table 4 shows precision across operators.

Table 2: Within Lab Precision Panel

Panel Member	Level Tested	GBS (CFU/mL)
Moderate Positive (MP)	3-4x LoD	1600
Low Positive (LP)	1-2x LoD	600
Moderate Negative (MN)	>10-fold dilution of 1x LoD	40
Low Negative (LN)	>100-fold dilution of 1x LoD	4
True (Blank) Negative (TN)	0	0

Table 3: Qualitative Results from Within-Lab Precision Study (Across Instruments)

	Instrument 1	Instrument 2	Instrument 3	Overall
Level	Percent Positive	Percent Positive	Percent Positive	Percent Positive
MP	100% (72/72)	100%(72/72)	100%(72/72)	100% (216/216)
LP	100% (72/72)	95.8% (69/72)	97.2% (70/72)	97.7% (211/216)
	Percent Negative	Percent Negative	Percent Negative	Percent Negative
MN	77.7% (56/72)	86.1% (62/72)	83.3% (60/72)	82% (178/216)
LN	97.2% (70/72)	100% (72/72)	98.6% (71/72)	98.6% (213/216)
TN	100% (72/72)	100% (72/72)	100% (72/72)	100% (216/216)

Table 4: Quantitative GBS Parameter Analysis from Within Lab Precision (Across Operators)

	First Operator			Second Operator			Combined Data Set								
Level	Detected Pos/Total	% Positive	Ave Ct	Std Dev	% CV*	Detected Pos/Total	% Positive	Ave Ct	Std Dev	% CV	Detected Pos/Total	% Positive	Ave Ct	Std Dev	% CV
MP	108/108	100.0%	31.61	0.54	1.7%	108/108	100.0%	32.22	0.51	1.6%	216/216	100.0%	31.91	0.61	1.9%
LP	106/108	98.1%	34.16	0.68	2.0%	105/108	97.2%	34.39	0.72	2.1%	211/216	97.7%	34.27	0.71	2.1%
MN	20/108	18.5%	35.00	0.53	1.5%	18/108	16.7%	35.28	0.40	1.1%	38/216	17.6%	35.10	0.49	1.4%
LN	2/108	1.9%	35.49	0.12	0.3%	1/108	0.9%	35.03	N/A		3/216	1.4%	35.33	0.28	0.8%
TN	0/108	0.0%	N/A			0/108	0.0%	N/A			0/216	0.0%	N/A		

%CV: The coefficient of variation, 100\* standard deviation/Ave Ct.

#### Reproducibility

#### Inter-Lab Reproducibility

The reproducibility of the NeuMoDx™ GBS Assay as implemented on the NeuMoDx™ 288 Molecular System using the NeuMoDx™ GBS Test Strip was evaluated at 3 different testing sites by testing 5 replicates of a 4-member panel over 5 days, which generated a total of 75 replicates per panel member. Panel samples were prepared by spiking cultured GBS into pooled, negative clinical Lim broth to create Low Negative, Low Positive and

Moderate Positive panel members, whereas the True (Blank) Negative samples contained no GBS. Concentrations of the panel members correspond to the same levels listed in Table 8 above used for Precision (minus the Moderate Negative sample). A positive and a negative external control were also processed on each day of testing.

Overall, there were 4 invalid results obtained during the Reproducibility study – one replicate of each of the 4 concentrations yielded an "Indeterminate" and all occurred on the same day of testing (Day 2) at Site B. Upon repeat testing, 2 of the 4 samples yielded a valid, correct result; the remaining two samples yielded an "Indeterminate" result a second time before yielding a valid, correct result. The percent agreement with the expected result for the panel members for all sites combined is presented in Table 5.

Table 5: Inter-Lab Reproducibility Performance Summary of the NeuMoDx™ GBS Assay

Panel Member Concentration	Site 1 (A)	Site 2 (B)	Site 3 (D)	Total Agreement (CI 95%) <sup>a</sup>
Moderate Positive	25/25	25/25	25/25	100% (75/75) (95.1 – 100)
Low Positive	24/25	25/25	24/25	97.3% (73/75) (90.8 – 99.3)
Low Negative	25/25	25/25	24/25 b	98.7% (74/75) (92.8 – 99.8)
Blank Negative	25/25	25/25	25/25	100% (75/75) (95.1 – 100)

<sup>&</sup>lt;sup>a</sup> The lower and upper limits of the presented 95% confidence interval (CI) were calculated using the 95% score confidence interval method.

#### b. Linearity/Assay Reportable Range

Not applicable. The NeuMoDx™ GBS Assay is a qualitative test

#### c. Traceability, Stability, Expected Values

#### **Traceability**

Traceability to a certified control or calibrator is not applicable as there are no certified external controls or calibrators available for use with GBS assays. NeuMoDx developed internal sample processing controls, included in the assay reagents, to assure test methods were properly executed. The NeuMoDx $^{\text{TM}}$  GBS Assay Instructions for Use (IFU) contains a recommendation for external controls.

Product Lot and Serial Number traceability has been implemented through the use of Unique Device Identifier (UDI) and GS1 compatible 2D and 1D barcodes within the unit labeling. The GBS Test Strip as used for the GBS Assay contains a (device) serial number for each unit/piece.

#### Stability

Stability studies were performed to assess the in-use stability of the reagents and the shelf-life stability of the packaged NeuMoD $x^{TM}$  GBS Test Strip and the Lysis Buffer 4.

<sup>&</sup>lt;sup>b</sup> The Low Negative sample concentration is anticipated to be detected as positive ~5% of the time.

#### d. Detection Limit

The Analytical Sensitivity of the NeuMoDx<sup>™</sup> GBS Assay using the NeuMoDx<sup>™</sup> GBS Test Strip was characterized by testing five different levels of GBS (ATCC BAA-611 serotype V) prepared from five independent clinical negative pools on the NeuMoDx<sup>™</sup> 288 Molecular System.

The study was performed over non-consecutive days across multiple systems with each system processing ten replicates at each level per day. A unique lot of each of the following: NeuMoDx™ GBS Test Strip, NeuMoDx™ Extraction Plate and NeuMoDx™ Lysis Buffer 4 was tested on each System. Detection rates are depicted in the following table. The LoD was determined to be 500 CFU/mL.

Positive percent detection rates for samples used to determine LoD of the NeuMoDx™ GBS Assay

GBS CFU/mL	Number of Valid Tests	Number of Positives	Number of Negatives	Detection Rate	
1000	60	60	0	100%	
500*	60	60	0	100%	
200	60	53	7	88%	
100	60	35	25	58%	
0	60	0	60	0%	

<sup>\*</sup>equivalent to 20 CFU/test

#### e. Analytical Reactivity (Inclusivity)

The NeuMoDx<sup>™</sup> GBS Assay as implemented using the NeuMoDx<sup>™</sup> GBS Test Strip detected all major serotypes of group B S*treptococcus*, including the four most clinically relevant. The twelve different strains of GBS bacteria spanning the serotypes that were tested using the NeuMoDx<sup>™</sup> GBS Test Strip are shown in Table 6.

Table 6: GBS Serotypes Tested

able 6. GBS Selectypes Tested						
GBS Serotype	GBS Strain	ATCC/BEI#	Concentration (CFU/mL) with 100% Detection			
la	A909	ATCC: BAA-1138	1500			
lb	H36b	ATCC: BAA-1174	1000			
II	MNZ933	BEI: NR-43896	400			
III	MNZ938	BEI: NR-43897	400			
lc	CDC SS700	ATCC: 27591	800			
IV	2011201884	ATCC: BAA-2673	800			
VI	2010228816	ATCC: BAA-2671	800			
VII	4832-06	ATCC: BAA-2670	800			
VIII	5030-08	ATCC: BAA-2669	800			
IX	7509-07	ATCC: BAA-2668	800			
Non-hemolytic	NCTC 8181	ATCC: 13813	800			
TX Clinical Isolate 2012	SGBS030	BEI: NR-44144	800			

#### f. Analytical Specificity (Exclusivity)

#### **Analytical Specificity and Cross-reactivity**

Analytical specificity was demonstrated by screening 136 organisms common to the urogenital and digestive tract, as well as species phylogenetically related to GBS for cross-reactivity on the NeuMoDx<sup>TM</sup> 288 Molecular System using the NeuMoDx<sup>TM</sup> GBS Test Strip. Organisms were prepared in pools of 5-6 and tested at a high concentration (bacteria 6 –  $9\times10^6$  CFU/mL; viruses  $1\times10^6$ - $1\times10^7$  copies/mL).

None of the organisms screened demonstrated cross-reactivity when implementing the NeuMoDx $^{\text{TM}}$  GBS Assay. The organisms tested are shown in Table 7.

Table 7: Pathogens Used to Demonstrate Analytical Specificity

Bacteria, Yeast and Parasites					
Streptococcus pyogenes	Salmonella enterica (serovar Minnesota)	Cryptococcus neoformans			
Streptococcus salivarius	Alcaligenes faecalis	Candida glabrata			
Streptococcus sanguinis	Staphylococcus saprophyticus	Achromobacter xerosis			
Moraxella (Branhamella) catarrhalis	Eikenella corrodens	Rhodospirillum rubrum			
Neisseria gonorrhoeae	Enterococcus avium	Neisseria subflava			
Streptococcus pyogenes	Micrococcus luteus	Pseudomonas putida			
Streptococcus mitis	Citrobacter freundii	Bacillus subtilis			
Lactococcus lactis;	Gemella haemolysans	Corynebacterium xerosis			
Listeria monocytogenes	Kingella kingae	Mycobacterium smegmatis			
Morganella morganii	Rahnella aquatilis	Legionella pneumophila			
Plesiomonas shigelloides	Bacillus cereus	Moraxella lacunata			
Proteus vulgaris	Aeromonas hydrophila	Streptomyces griseus			
Salmonella enterica (serovar Typhi)	Enterobacter cloacae	Gardnerella vaginalis			
Staphylococcus aureus	Brevibacterium linens	Clostridium perfringens			
Staphylococcus epidermidis	Candida parapsilosis	Peptostreptococcus anaerobius			
Streptococcus mutans	Lactobacillus brevis	Bifidobacterium adolescentis			
Yersinia enterocolitica	Deinococcus radiodurans	Derxia gummosa			
Providencia stuartii	Pseudomonas protegens	Veillonella parvula			
Pseudomonas aeruginosa	Acinetobacter calcoaceticus	Mycoplasma pneumoniae			
Acinetobacter lwoffii	Lactobacillus acidophilus	Bacteroides fragilis			
Proteus mirabilis	Vibrio parahaemolyticus	Acinetobacter baumannii			
Klebsiella pneumoniae	Corynebacterium genitalium	Corynebacterium,strain HFH0082			
Aerococcus viridans	Enterococcus faecalis	Enterobacter aerogenes			
Enterococcus faecium	Salmonella enterica	Klebsiella oxytoca			
Neisseria lactamica	Lactobacillus jensenii	Escherichia coli			
Neisseria meningitidis	Lactobacillus delbrueckii	Streptococcus canis			
Streptococcus pneumoniae	Serratia marcescens	Streptococcus dysgalactiae			
Kingella denitrificans	Candida albicans	Streptococcus oralis			
Haemophilus influenzae	Candida tropicalis	Streptococcus uberis			
Neisseria perflava	Chromobacterium violaceum	Streptococcus suis			
Moraxella osloensis	Candida krusei				
Neisseria meningitidis Sero C	Saccharomyces cerevisiae				
Neisseria meningitidis Sero A	Corynebacterium urealyticum	Viruses			
Streptococcus anginosus (Grp C)	MRSA	CMV*			
Streptococcus bovis	Chlamydia trachomatis	EBV (HHV-4)			
Streptococcus intermedius	Bifidobacterium breve	HSV1*			
Neisseria meningitidis M158 group D	Mobiluncus mulieris*	HSV2*			
Neisseria flavescens	Propionibacterium acnes	VZV (HHV 3)*			
Streptococcus parasanguinis	Campylobacter jejuni	HPV-16*			
Lactobacillus casei	Haemophilus ducreyi	JC virus*			
Lactobacillus lactis	Mycoplasma hominis	BK virus			
Haemophilus influenzae type B	Mycoplasma genitalium	HHV-6A			
Salmonella newport	Trichomonas vaginalis	HHV-6B			
Shigella flexneri	Pseudomonas fluorescens	HHV-7			
Shigella sonnei	Enterococcus dispar	HHV-8			
Enterococcus durans	Ureaplasma urealyticum				
Enterococcus sp. (ATCC® 202155™)	Chlamydia pneumoniae*				
*Tested at 10 ng/ml					

<sup>\*</sup>Tested at 10 ng/ml

#### g. Interference with Non-Target Organisms

The NeuMoDx™ GBS Assay was tested for interference in the presence of non-target organisms (co-habiting in the urogenital tract) by evaluating the performance of the assay at low levels of GBS on the NeuMoDx™ 288 Molecular System. The same panel of 136 organisms (Table 6) used for assessing cross-reactivity was used for this study. The organisms were pooled into groups of 5-6 in clinical negative Lim broth and spiked with 1200 CFU/mL cultured GBS. Testing validated detection of group B streptococcus in all of the pools tested. No interference due to commensal organisms was observed.

#### h. Interference with Exogenous and Endogenous Substances

The performance of the NeuMoDx<sup>™</sup> GBS Assay was assessed on the NeuMoDx<sup>™</sup> 288 Molecular System in the presence of exogenous and endogenous interfering substances which may typically be encountered in GBS clinical specimens. Each of the endogenous and exogenous substances listed, in Table 8, were added to pooled clinical negative Lim broth samples containing GBS at 1200 CFU/mL or 4000 CFU/mL. The 20 exogenous and 6 endogenous substances that were tested for interference using the NeuMoDx<sup>™</sup> GBS Test Strip resulted in no adverse effect on detection of GBS at either level tested further demonstrating the robustness of the NeuMoDx<sup>™</sup> GBS Assay.

Table 8: Exogenous and Endogenous Interfering Agents tested

Ex	ogenous Substances		Endogenous Substances
Monistat® Cream	Dulcolax® Suppositories	K-Y™ Jelly	Human Amniotic Fluid
Yeast Gard Advanced™ (Douche)	Fleet® Enema	McKesson Gel	Human Whole Blood
Metamucil® Fiber Supplement	Preparation H <sup>®</sup> Cream	Contraceptive Foam	Human Urine
Ex-lax <sup>®</sup> (Chocolate Pieces)	Vagisil™ Powder	Moisturizing Lotion	Human Fecal Sample
Phillips'® Milk of Magnesia	Norforms® Suppositories	Neutrogena® Body oil	Mucus
Pepto-Bismol™	FDS® Deodorant Spray	Gold Bond® Powder	Human Genomic DNA
Kaopectate®	New Mama Bottom Spray		

#### i. Carry-Over and Cross-Contamination Studies

Potential sample carry-over and cross-contamination studies were performed on the NeuMoDx<sup>™</sup> 288 Molecular System using the NeuMoDx<sup>™</sup> GBS Test Strip. The two-part study first evaluated the impact on GBS negative samples by being interspersed with samples containing high GBS target (at 1x10<sup>7</sup> CFU/mL). The positive and negative samples were loaded such that each negative sample was adjacent to a high positive sample. The second part of this study processed all negative samples immediately following a run which had processed all high GBS concentration samples.

No contamination was seen in negative samples integrated with high level samples, or in negative samples that followed samples with high concentrations of GBS demonstrating the lack of any carry over and / or cross-contamination.

#### 8. COMPARISON STUDIES

- a. Method Comparison with Predicate Device Not Applicable
- **b. Matrix Comparison**Not Applicable

#### 9. CLINICAL PERFORMANCE SUMMARY

#### **Clinical Performance**

Performance characteristics were determined during a prospective clinical method comparison study conducted at three (3) geographically diverse laboratory locations to evaluate the comparative performance of the of the NeuMoDx™ GBS Assay as implemented on the NeuMoDx™ 288 Molecular System compared to conventional culture methods recommended by the Center for Disease Control (CDC) to identify GBS from subcultures of enriched Lim broth. Specimens eligible for enrollment were collected from pregnant women by health care providers for routine standard of care screening purposes recommended by the CDC between 35-37 weeks gestation.

The collected vaginal / rectal swab specimens were transported to the various laboratories in appropriate transport medium and then inoculated into a selective Lim broth medium by laboratory personnel in preparation to undergo an 18 - 24 hour incubation period. Following the incubation period and routine care testing, the residual Lim broth samples were subcultured to a sheep blood agar plate as recommended by the 2010 published CDC procedures for processing clinical specimens for culture of GBS. The agar plates were incubated for up to 48 hours and inspected for organisms suggestive of GBS. Suspected colonies were Gram-stained and the Gram-positive cocci colonies were tested for catalase production; Gram positive cocci colonies that tested negative for catalase production were worked-up for further identification by a streptococcal grouping latex agglutination test to determine the presence of GBS. Clinical performance is based on 1193 specimens with complete, valid, and compliant results included in the study and summarized in the tables below. The lower and upper limits of the presented 95% confidence interval (CI) were calculated using the 95% score confidence interval method.

NeuMoDx™ GBS Assay Clinical Performance Summary

		Culture / Reference Method			
Clinical Site	Summary	Positive	Negative	Total	
No. Ma Do	Positive	253	37	290	
NeuMoDx ™ GBS	Negative	8	895	903	
	Total	261	932	1193	

Sensitivity = 96.9% 95% CI (94.1 – 98.4) Specificity = 96.0% 95% CI (94.6-97.1)

Site Specific Clinical Performance of the NeuMoDx™ GBS Assay

Site	n	Sensitivity (95% CI) <sup>a</sup>	Specificity (95% CI) <sup>a</sup>	Prevalence <sup>b</sup> (95% CI) <sup>a</sup>
Α	351	92.4% 73/79 (84.4-96.5)	96.7% 263/272 (93.8-98.3)	22.5% 79/351 (15.1-22.2)
В	400	98.4% 62/63 (91.5-99.7)	94.4% 318/337 (91.4-96.4)	15.8% 63/400 (10.8-17.0)
С	442	99.2% 118/119 (95.4-99.9)	97.2% 314/323 (94.8-98.5)	26.9% 119/442 (18.2-24.7)
Total	1193	96.9% 253/261 (94.1-98.4)	96.0% 895/932 (94.6-97.1)	21.9% 261/1193 (19.6-24.3)

<sup>&</sup>lt;sup>a</sup> The lower and upper limits of the presented 95% confidence interval (CI) were calculated using the 95% score confidence interval method.

#### 9. INSTRUMENT NAME

NeuMoDx™ 288 Molecular System

#### 10. SYSTEM SOFTWARE

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes	Χ	No

### 11. CONCLUSIONS DRAWN FROM NON-CLINICAL AND CLINICAL TESTS

The subject device and the predicate device are substantially equivalent, with respect to intended use, instructions for use, design features, technological characteristics, manufacturing methods, performance criteria, special controls, and safety and effectiveness. The subject device is substantially equivalent to the predicate device (K090191).

#### 12. CONCLUSION

Based on the information contained herein, we conclude the NeuMoDx<sup>™</sup> GBS Assay (subject device) when implemented on the NeuMoDx<sup>™</sup> 288 Molecular System is substantially equivalent to the legally marketed predicate device (K090191), and is safe and effective for its labeled intended use.

<sup>&</sup>lt;sup>b</sup> Prevalence calculations based on reference method results obtained by following the CDC-recommended procedures for processing clinical specimens for culture of group B *Streptococcus*. (Published 2010)

# EXHIBIT 24

# (12) United States Patent Williams et al.

# (10) Patent No.: US 9,050,594 B2

# (45) **Date of Patent: Jun. 9, 2015**

#### (54) SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

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(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 127 days.

(21) Appl. No.: 13/766,359

(22) Filed: Feb. 13, 2013

(65) **Prior Publication Data** 

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#### Related U.S. Application Data

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(51) **Int. Cl.** *C12M 1/34 C12M 3/00*(2006.01)

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(52) U.S. Cl.

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#### 58) Field of Classification Search

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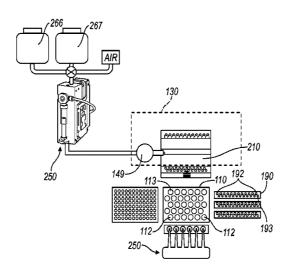
Primary Examiner — Nathan Bowers

Assistant Examiner — Lydia Edwards
(74) Attorney, Agent, or Firm — Jeffrey Schox; Ivan Wong

#### (57) ABSTRACT

A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.

#### 32 Claims, 24 Drawing Sheets

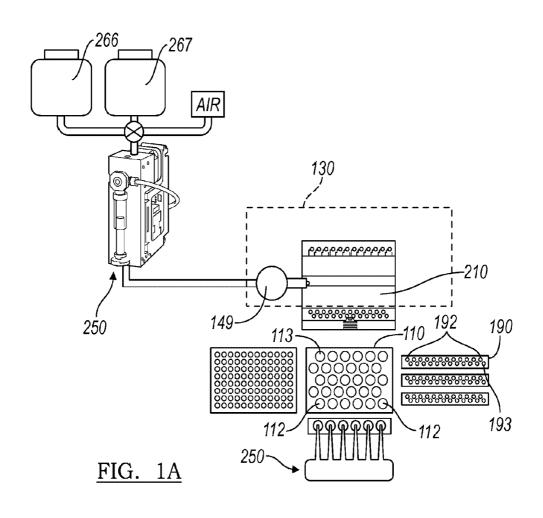


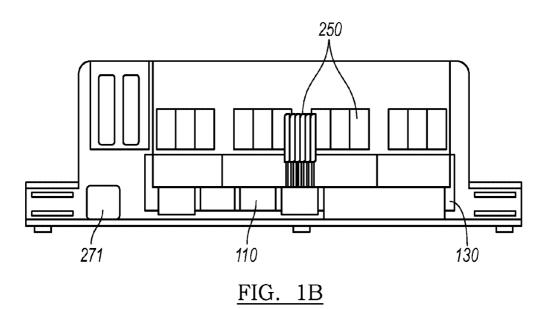
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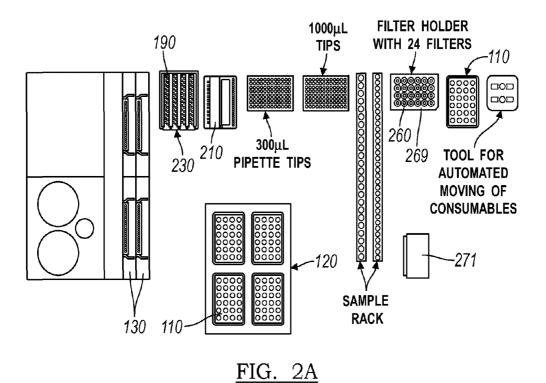
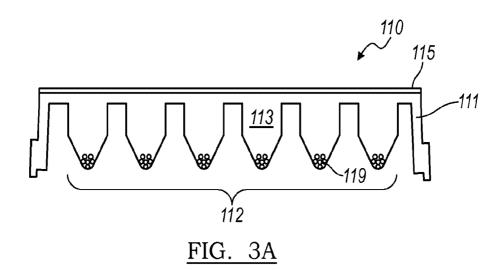
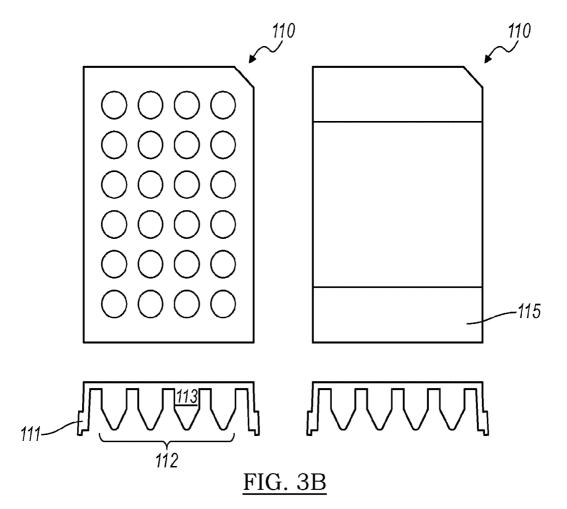


FIG. 2B

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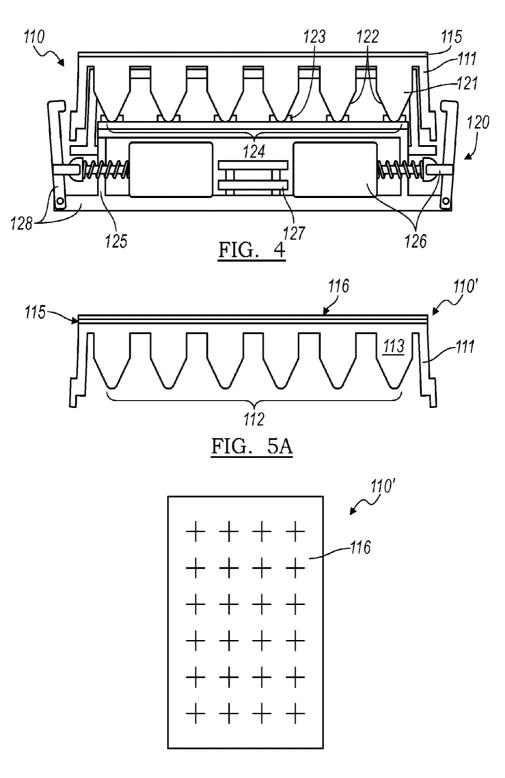
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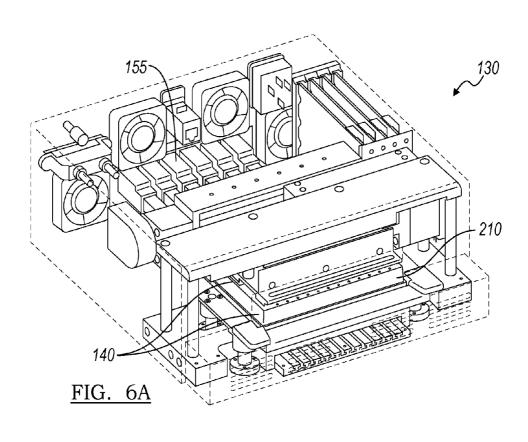
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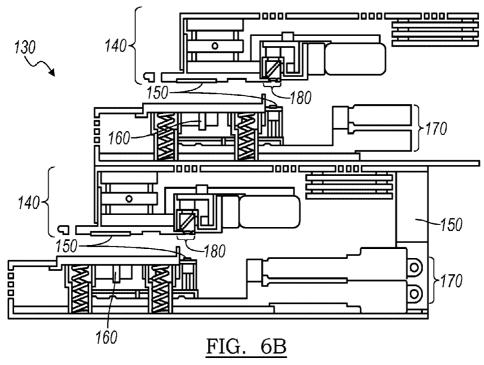


<u>FIG. 5B</u>

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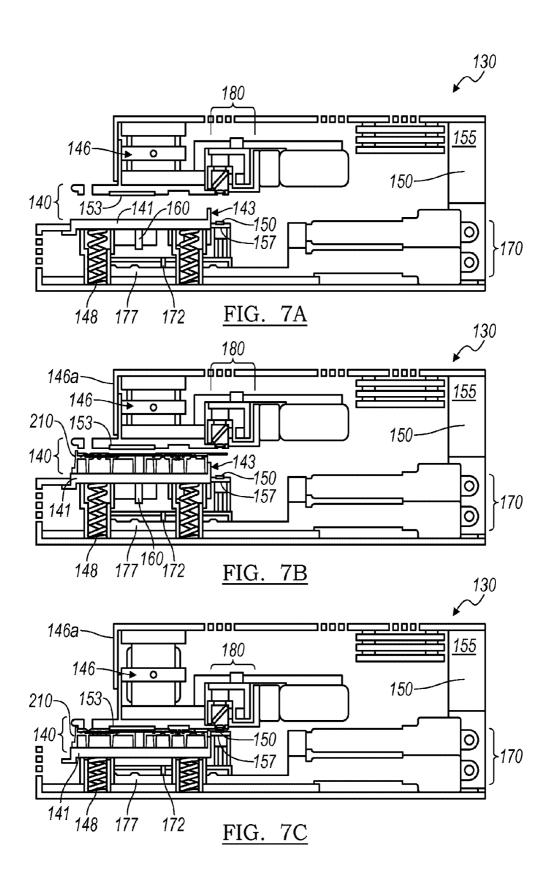
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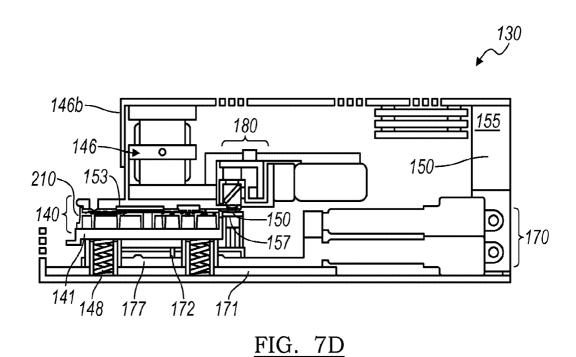
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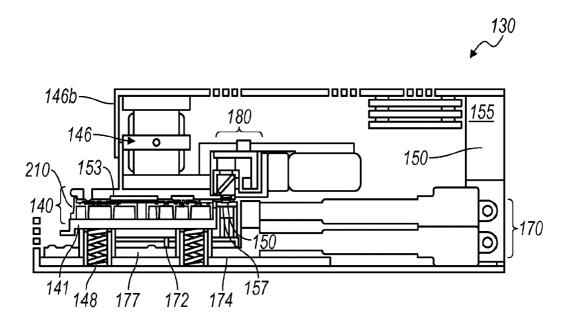
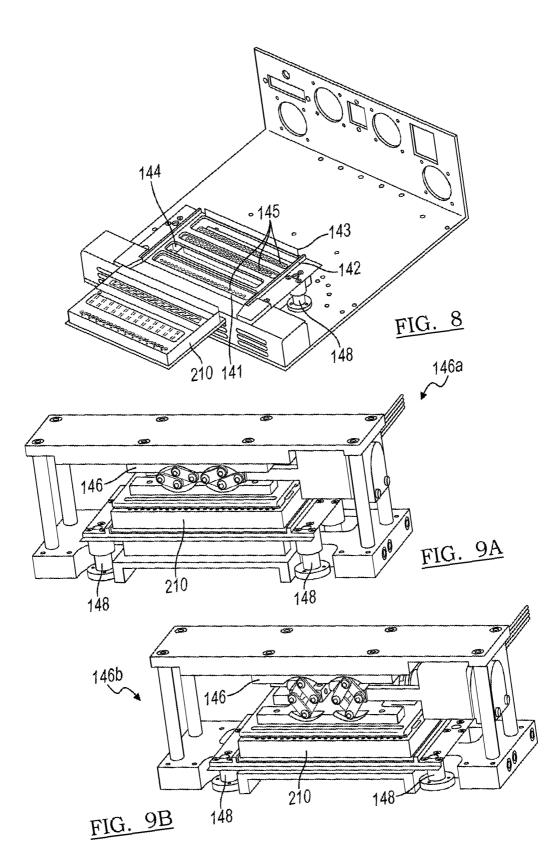


FIG. 7E

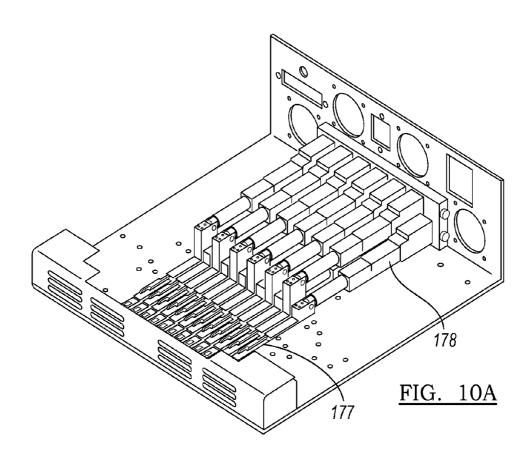
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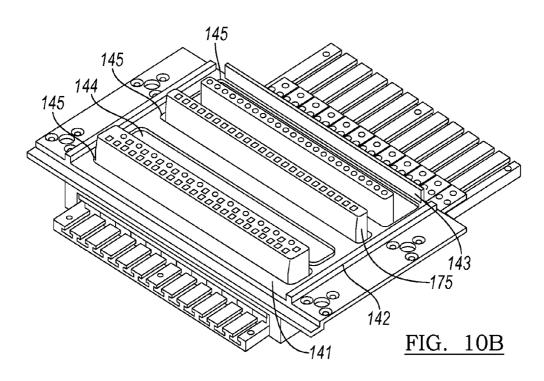


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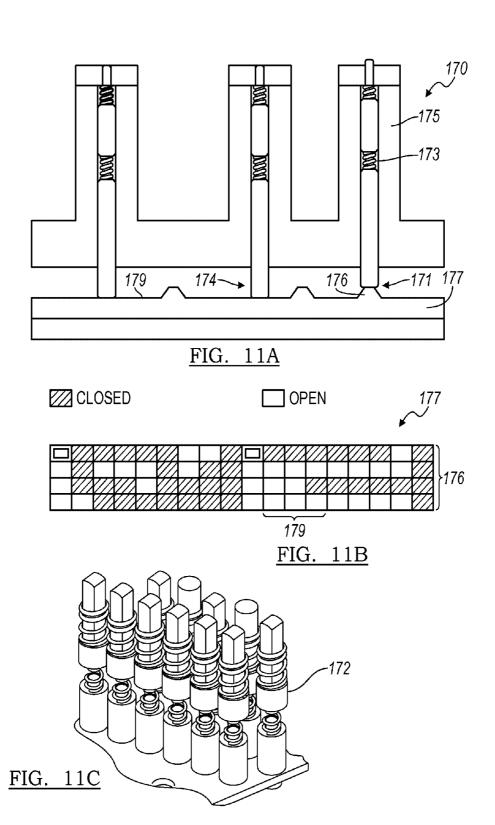
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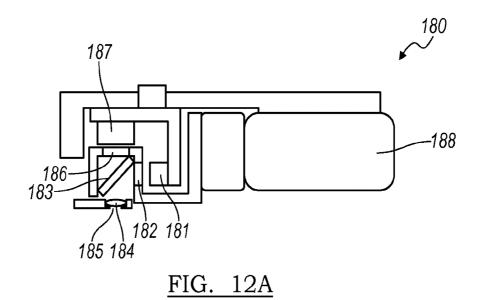
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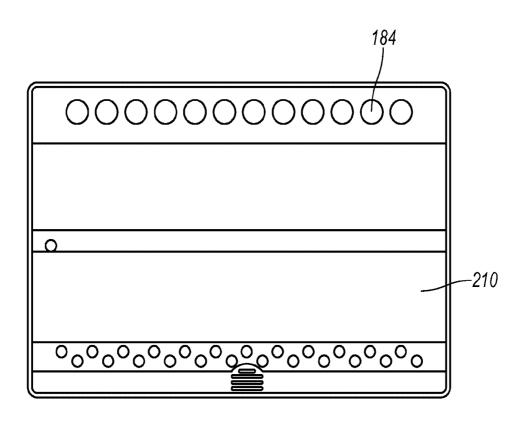
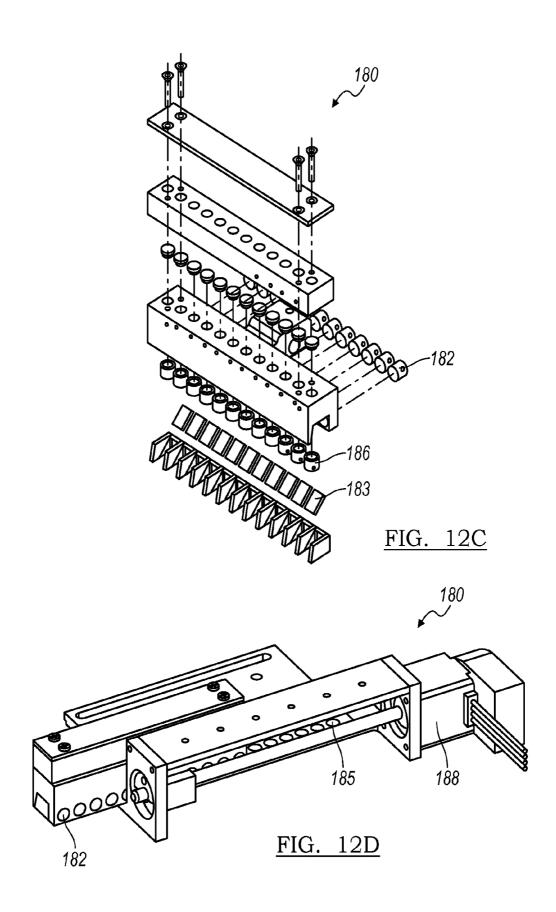


FIG. 12B

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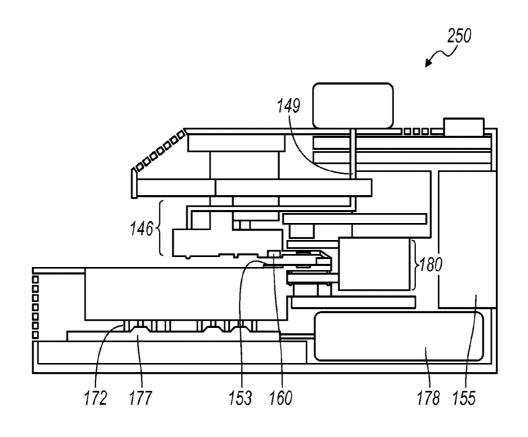
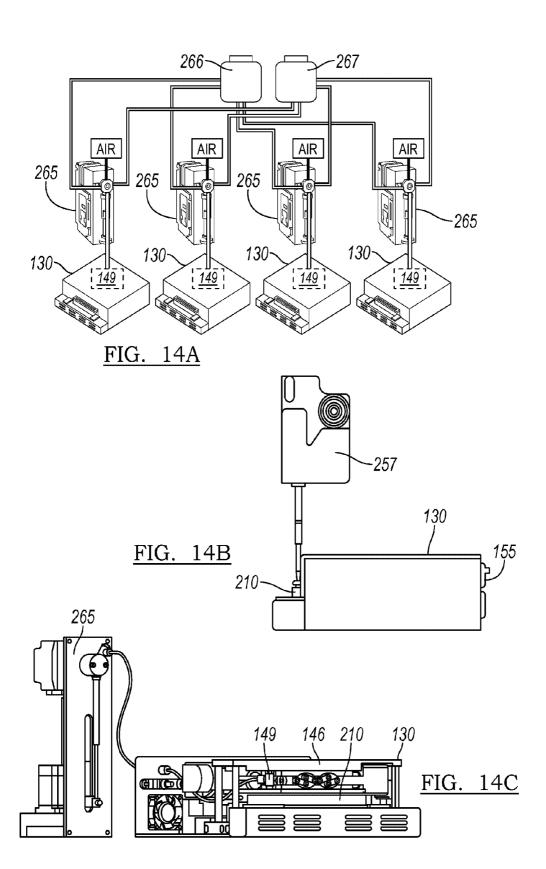


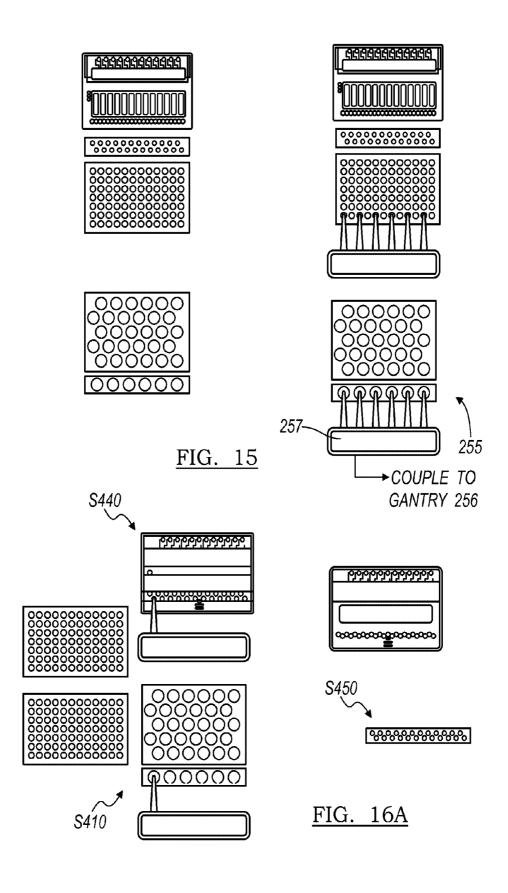
FIG. 13

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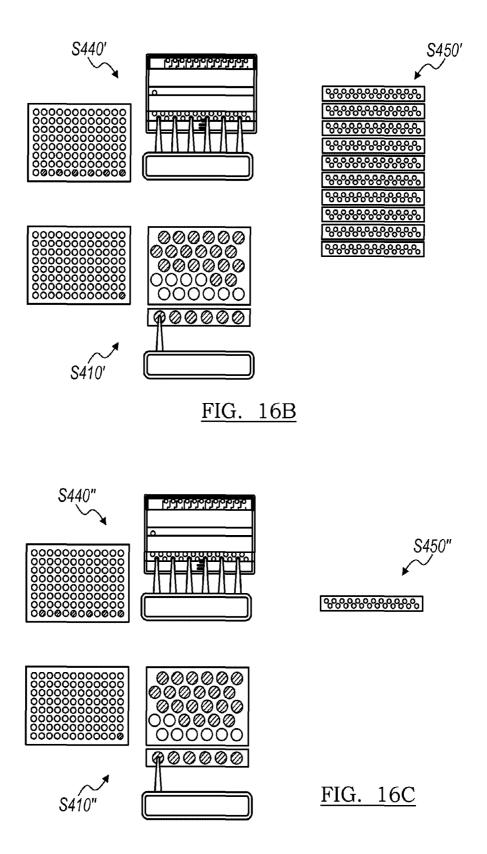
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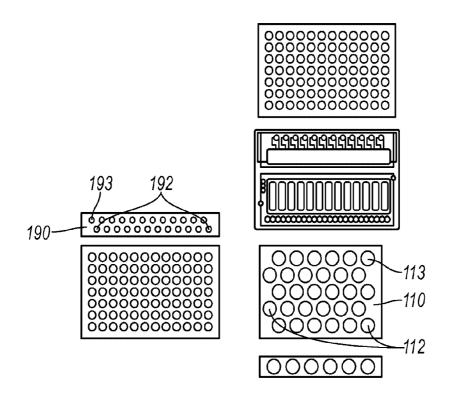
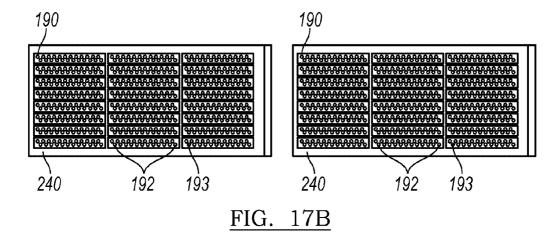
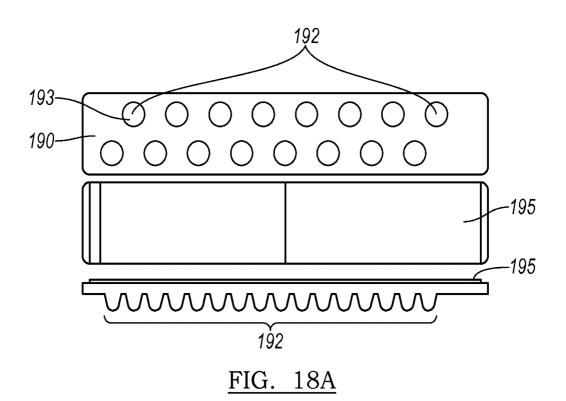


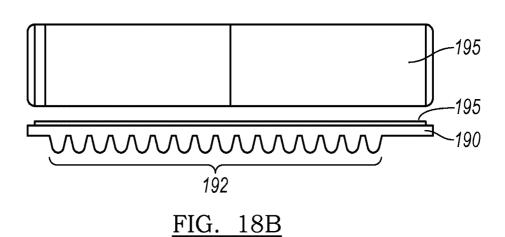
FIG. 17A



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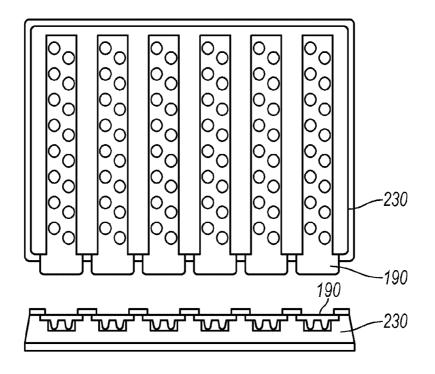
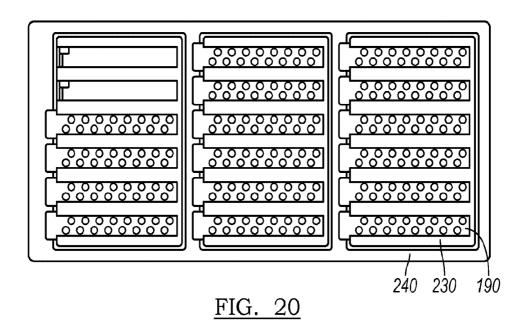
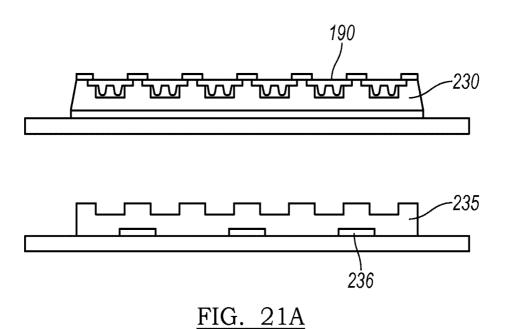


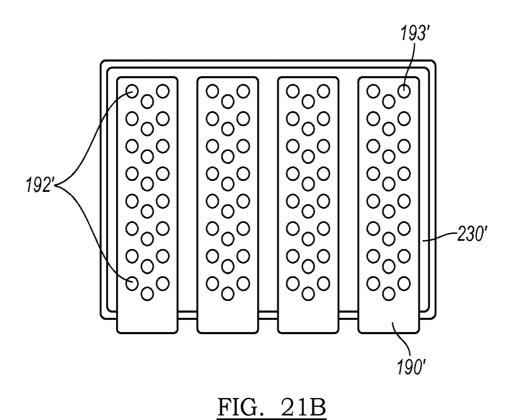
FIG. 19



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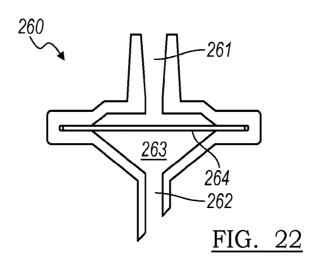
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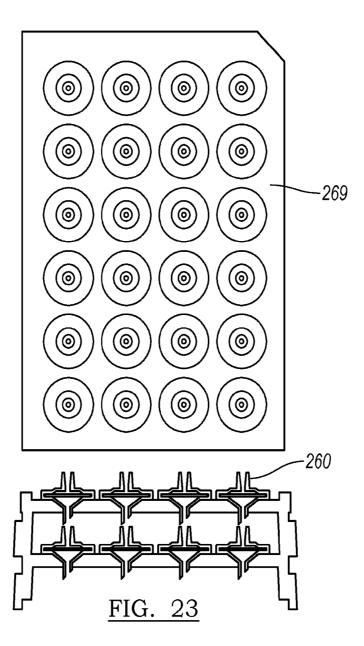




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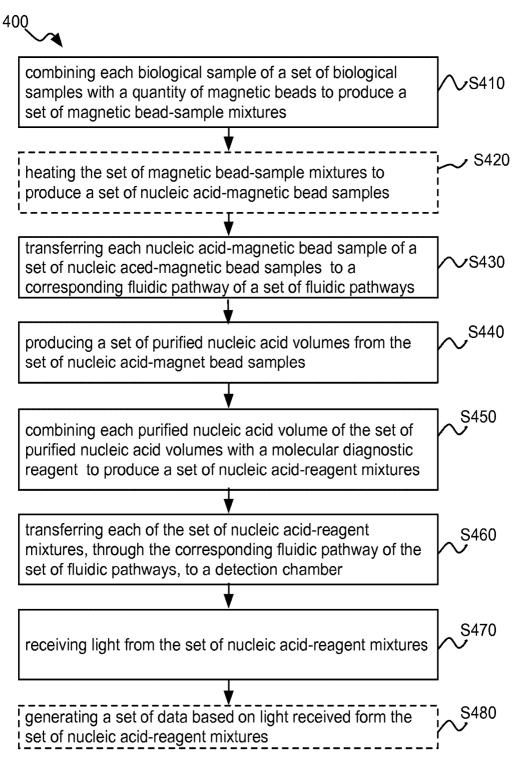


FIG. 24A

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s430

transferring each nucleic acid-magnetic bead sample of a

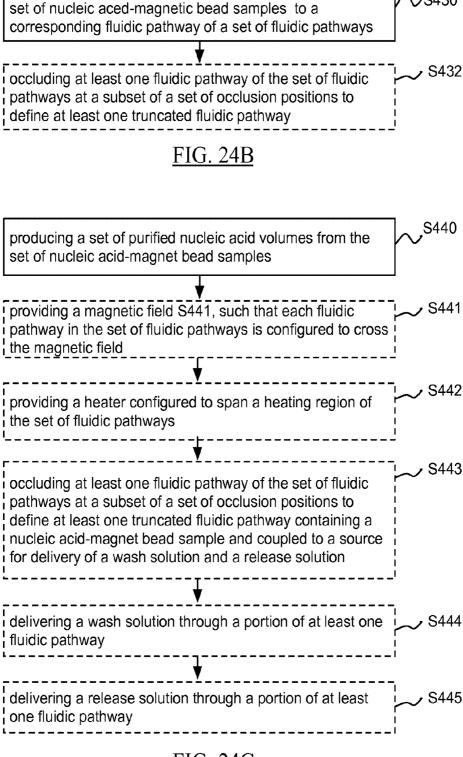


FIG. 24C

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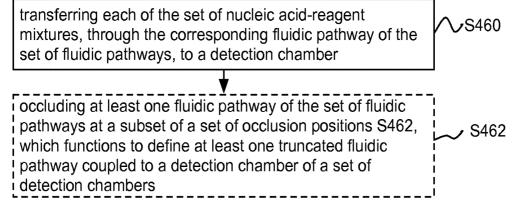


FIG. 24D

#### SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

#### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are incorporated herein in their entirety by  $\ ^{10}$ this reference. This application is also related to U.S. application Ser. No. 13/765,996, which is incorporated herein in its entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved system and method for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a clinical laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology 25 research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields involving analysis of nucleic acids. Molecular diagnostic 30 analysis of biological samples can include the detection of one or more nucleic acid materials present in the specimen. The particular analysis performed may be qualitative and/or quantitative. Methods of analysis typically involve isolation, purification, and amplification of nucleic acid materials, and 35 polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/ or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecu- 40 lar diagnostic techniques are often labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are specific to certain sample matrices and/or and nucleic acid types.

Due to these and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for and improved system and method for processing and detecting nucleic acids. This invention provides such a system and 50 method.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1B depict an embodiment of a system for pro- 55 cessing and detecting nucleic acids;

FIGS. 2A-2B depict an embodiment of elements, and a top view of an embodiment of a system worktable, respectively, of an embodiment of a system for processing and detecting nucleic acids;

FIGS. 3A-3B depict an embodiment of a capture plate for combining a sample with magnetic beads;

FIG. 4 depicts an embodiment of a capture plate module to facilitate lysis of a biological sample and combination of the biological sample with magnetic beads;

FIGS. 5A-5B depict an alternative embodiment of a capture plate;

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FIGS. 6A-6B depict embodiments of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 7A-7E depict a sequence of operations performed by elements of an embodiment of a molecular diagnostic mod-

FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform;

FIGS. 9A-9B depict configurations of a linear actuator of an embodiment of a molecular diagnostic module;

FIGS. 10A-10B depict elements of an embodiment of a valve actuation subsystem of a molecular diagnostic module;

FIGS. 11A-11C depict an embodiment of a valve actuation subsystem of a molecular diagnostic module;

FIGS. 12A-12D depict elements of an embodiment of an 15 optical subsystem of a molecular diagnostic module;

FIG. 13 depicts a side view of an alternative embodiment of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 14A-14C depict an embodiment of a fluid handling 20 system of a system for processing and detecting nucleic acids; FIG. 15 depicts embodiments of elements of the fluid handling system;

FIGS. 16A-16C are schematics depicting example methods for processing and detecting nucleic acids;

FIGS. 17A-17B show embodiments of consumables and reagents used in a system for processing and detecting nucleic acids;

FIGS. 18A-18B depict an embodiment of an assay strip to facilitate analysis of a sample containing nucleic acids;

FIG. 19 depicts an embodiment of an assay strip holder; FIG. 20 depicts an embodiment of an assay strip carrier; FIGS. 21A-21B show alternative embodiments of assay strip holders and assay strips, respectively;

FIG. 22 shows an embodiment of a filter to facilitate processing and detecting of nucleic acids;

FIG. 23 shows an embodiment of a filter holder to facilitate processing and detecting of nucleic acids; and

FIGS. 24A-24D depict embodiments of a method for processing and detecting nucleic acids.

#### DESCRIPTION OF THE PREFERRED **EMBODIMENTS**

The following description of preferred embodiments of the nucleic acid types and not applicable across common sample 45 invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

1. System for Processing and Detecting Nucleic Acids

As shown in FIGS. 1A-1B and 7A, an embodiment of a system 100 for processing and detecting nucleic acids comprises: a capture plate 110 configured to facilitate binding of nucleic acids within a biological sample to a set of magnetic beads 119; a molecular diagnostic module 130 comprising a microfluidic cartridge receiving module 140, heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, an optical subsystem 180; and an assay strip 190 configured to facilitate mixing of molecular diagnostic reagents with a nucleic acid volume. Other embodiments of the system 100 may further comprise at least one of a capture 60 plate module 120 configured to support the capture plate 110; a filter 200 and filter holder 205 to facilitate sample preparation; a microfluidic cartridge 210 configured to facilitate sample processing; an assay strip holder 230; an assay strip carrier 240; a liquid handling system 250 configured to facilitate gas and fluid delivery to different elements of the system 100; a processor configured to analyze data resulting from a run of the system 100; and a user interface configured to allow

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a user to interact with the system 100. The system 100 thus functions to receive biological samples containing nucleic acids (i.e., impure nucleic acid samples), separate nucleic acids from the biological samples, and analyze nucleic acid samples according to at least one molecular diagnostic protocol (e.g., PCR). Preferably, the system 100 is a walkaway system by which a user loads a set of biological samples containing nucleic acids, and receives a set of data resulting from a molecular diagnostic protocol without any further sample manipulation by the user. Alternatively, the system 100 facilitates aspects of sample preparation for a molecular diagnostic protocol, with some sample manipulation performed by the user.

In one example workflow of the system 100, a liquid handling system 250 aspirates a set of biological samples con- 15 taining nucleic acids (i.e., impure nucleic acid samples), and dispenses the set of biological samples into a capture plate 110 to be lysed and combined with magnetic beads (containing a proprietary affinity coating to bind the nucleic acids to the magnetic beads) by a capture plate module 120. The liquid 20 handling system 250 then aspirates substantially all of each sample of the set of lysed biological samples combined with magnetic beads (i.e., set of magnetic bead-samples) from the capture plate no, and dispenses the set of magnetic beadsamples into a microfluidic cartridge 210, aligned within a 25 cartridge receiving module 140 of a molecular diagnostic module 130, and configured to be manipulated by the molecular diagnostic module 130. A heating and cooling subsystem 150, a magnet 160, and a valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate separation of 30 a set of nucleic acids from the magnetic bead-samples, as the liquid handling system 250 dispenses wash solutions, release solutions, and/or air at appropriate stages. The liquid handling system 250 then aspirates the set of nucleic acids from the microfluidic cartridge 210 contained within the molecular 35 diagnostic module 130, combines the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, and dispenses the set of nucleic acids combined with the set of molecular diagnostic reagents (i.e., set of nucleic acidreagent mixtures) into the microfluidic cartridge 210 within 40 the molecular diagnostic module 130. The detection chamber heaters 157, optical subsystem 180 and valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user 45 interface.

As stated, the above workflow is just one example workflow of the system 100, and other workflows of the system 100 and methods of processing and detecting nucleic acid samples are further described in Section 2 below. A detailed 50 description of elements of an embodiment of the system 100 are described in sections 1.1-1.6 below.

#### 1.1 System—Capture Plate and Capture Plate Module

As shown in FIGS. 3A and 3B, the capture plate 110 comprises a capture plate substrate in comprising a set of 55 wells 112 and a puncturable foil seal 115, and functions to facilitate binding of nucleic acids within a biological sample to a set of magnetic beads 119. Preferably, the entire capture plate 110 is configured to be a consumable (i.e., disposable), such that each well of the capture plate 110 can only be used once yet the remaining unused wells can be used during additional runs of the system 100. Alternatively, at least a portion of the capture plate 110 is configured to be reusable, such that additional mixing or reagent additions can be performed and portions of the capture plate 110 may be used for 65 multiple runs of the system 100. In one variation of the capture plate 110, the capture plate substrate 111 is reusable,

while the puncturable foil seal 115 is disposable and replaced after each run of the system 100.

The capture plate substrate 111 is configured such that the capture plate 110 is capable of resting on a flat surface, can be stacked with another capture plate 110, and also can be manipulated with industry standard instrument components for handling of microtiter plates. The capture plate substrate also functions to define the set of wells 112 and to couple to the puncturable foil seal 115. The capture plate substrate in is preferably composed of a PCR-compatible polymer that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 112 of the capture plate substrate 111 function to receive at least one biological sample which contain or are suspected of potentially containing nucleic acids, and to facilitate combination of the biological sample with a set of magnetic beads 119. Preferably, the wells 113 are each configured to accommodate not only a biological sample, but also to facilitate mixing of the biological sample with a set of magnetic beads 119 (e.g., using a pipettor, the liquid handling system 250 or other apparatus), which preferably are preloaded in wells 112, or alternatively may be added by an operator. Preferably, the wells are also deeper than they are wide to allow a significant number of wells 112 (e.g. 24) with a clinically relevant sample volumes, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wider than they are deep to facilitate larger devices for mixing the biological samples with the magnetic beads 119. Each well 113 of the set of wells 112 also preferably has a conically shaped bottom region, as shown in FIG. 3A, to facilitate complete aspiration of a fluid from a well. Alternatively, each well 113 may not have a conically shaped bottom region. Additionally, in the orientation shown in FIG. 3A, the tops of each well 113 in the set of wells 112 preferably form raised edges protruding from the capture plate substrate 111, in order to facilitate sealing of each well 113 by the puncturable foil seal 115. Alternatively, the tops of each well 113 in the set of wells 112 may not form raised edges protruding from the capture plate substrate 111. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. However, the magnetic beads may alternatively be any appropriate magnetic beads (e.g. magnetic, parmagnetic, or superparamagnetic) configured to facilitate biomagnetic separation.

Each quantity of magnetic beads 119 may be accompanied by lysing reagents (e.g. proteinase K) and a sample process control comprising nucleic acid sequences for DNA and RNA, which function to lyse biological samples and to provide a mechanism by which sample process controls may be later detected to verify processing fidelity and assay accuracy. The sample process control comprising nucleic acid sequences for DNA and RNA allows one version of the capture plate to facilitate assays involving DNA and RNA detection. Preferably, the quantity of magnetic beads 119, lysing reagents, and sample process controls is dried within each well to improve shelf life; however, the quantity of magnetic beads 119, lysing reagents, and sample process controls may alternatively be in liquid form.

The puncturable foil seal 115 functions to isolate each well 113 of the set of wells 112, prevent contamination of the contents of each of the set of wells 112, protect the magnetic beads 119 and other reagents stored in wells 112 from deg-

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radation, and provide information identifying the capture plate no. The puncturable foil seal 115 preferably seals each well 113 of the capture plate 110, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one 5 variation, the puncturable foil seal 115 also forms a seal around an element that punctures it, and in another variation, the puncturable foil seal 115 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 115 is also preferably labeled with identifying information including at least one of manufacturer information, capture plate contents, the lot of the contents, an expiry date, and a unique electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal 115 does not extend beyond the footprint of the 15 capture plate no, but alternatively, the puncturable foil seal 115 may be any appropriate size and/or include protruding features (e.g., tabs) that facilitate handling of the capture

In one variation, the capture plate no may be prepackaged at least with magnetic beads 119, such that each well 113 in the set of wells 112 is prepackaged with a set of magnetic beads 119 defined by a specific quantity or concentration of magnetic beads. The set of wells 112 may then be sealed by the puncturable foil seal 115, which is configured to be punctured by an external element that delivers volumes of biological samples to be mixed with the magnetic beads 119. In another variation, the capture plate 110 may not be prepackaged with magnetic beads 119, but the wells 113 of the capture plate may still be sealed with a puncturable foil seal 115. In this variation, the puncturable foil seal 115 is configured to be punctured by at least one external element, for co-delivery of biological samples and magnetic beads intended to be combined.

A variation of the capture plate 110' may further comprise a slotted rubber membrane 116, as shown in FIGS. 5A and 5B, configured to provide access through the puncturable foil seal 115 to the set of wells 112. The slotted rubber membrane 116 thus functions to prevent or reduce splashing, evaporation, and/or aerosolization of contents of the set of wells 112. 40 Preferably, the slotted rubber membrane 116 comprises slots that are self-sealing and centered over wells of the set of wells 112, and further does not extend beyond the footprint of the capture plate 110. Alternatively, the slots of the slotted rubber membrane 116 may not be self-sealing, and/or the slotted rubber membrane 116 may be any appropriate size and comprise features that extend beyond the footprint of the capture plate 110.

In a specific example, the capture plate 110 comprises 24 wells 113 with an 18 mm center-to-center pitch, each well 50 having a volumetric capacity of 2 mL, and is compliant with Society for Laboratory Automation and Screening (SLAS) standards. Each well 113 of the capture plate no in the specific example is also prepackaged with a specified quantity of magnetic beads 119, and comprises a protruding top edge that 55 is heat sealed to a puncturable foil seal. In addition, each well 113 also contains other reagents beneficial for processing and monitoring the sample, including proteinase K and one or more specific nucleic acid stands designed to serve as a process control. The specific example of the capture plate 110 60 can thus combine two groups of 12 biological samples with magnetic beads. The capture plate 110 in the specific example is produced by injection molding, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier.

An embodiment of the system 100 may further comprise a capture plate module 120, as shown in FIG. 4, which func-

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tions to receive, support, and heat a capture plate 110. The capture plate module 120 preferably comprises a thermally conducting substrate 121 configured to cradle a capture plate 110, a capture plate heater 123, a capture plate receiving module 125, and a capture plate electronics module 127. Preferably, the capture plate module 120 functions to facilitate lysis of a biological sample deposited into a well 113 of the capture plate, and to facilitate binding of nucleic acids (i.e., within a lysed biological sample) to a quantity of magnetic beads 119 within a well 113 of the capture plate 110. In a specific example, the capture plate module 120 has dimensions of 108 mm×156 mm×45 mm and is configured to rest on a flat surface.

The thermally conducting substrate 121 is configured to cradle and support the capture plate 110, and functions to conduct heat to the set of wells 112 of the capture plate 110. Preferably, the thermally conducting substrate 121 is also configured to reversibly couple to the capture plate 110, and comprises a set of indentations 122 that encircle each well 113 in the set of wells 112. In one variation, the indentations 122 completely conform to the external surface of each well 113 of the capture plate 110, but in another variation, the indentations 122 may encircle a portion of each well 113 of the capture plate 110. Additionally, the indentations 122 are preferably thermally conducting in order to conduct heat to the set of wells 112, and portions of the thermally conducting substrate 121 aside from the indentations 122 are composed of non-conducting, rigid material. Alternatively, the entire thermally conducting substrate 121 may be composed of a material that is thermally conducting.

The capture plate heater 123 is preferably coupled to the thermally conducting substrate 121, and functions to transfer heat, through the thermally conducting substrate 121, to a well 113 of the capture plate 110. The capture plate heater 123 preferably conforms to at least a portion of an indentation 122 of the thermally conducting substrate 121, to facilitate heat transfer through the indentation 122 to an individual well 113 of the capture plate 110. In this variation, the capture plate heater 123 is one of a set of capture plate heaters 124, wherein each capture plate heater 123 in the set of capture plate heaters 124 transfers heat to an individual well 113 of the set of wells 112 of the capture plate 110. Alternatively, the capture plate heater 123 may conform to portions of multiple indentations 122 of the thermally conducting substrate 121, such that the capture plate heater 123 is configured to transfer heat to multiple wells 113 of the capture plate 110. Preferably, the capture plate heater 123 is a resistance heater, but alternatively, the capture plate heater 123 may be a Peltier or any appropriate heater configured to transfer heat to the capture plate 110. The capture plate heater 123 may also further couple to a heat sink.

The capture plate receiving module 125 comprises a capture plate actuation system 126 that functions to couple the capture plate module 120 to a capture plate 110. As shown in FIG. 4, the capture plate actuation system 126 comprises a structural support with hinged grips 128 and at least one capture plate module actuator 129. The capture plate module actuator 129 is preferably a push-type solenoid with a spring return, but may alternatively be any appropriate linear actuator, such as a hydraulic actuator. The structural support with hinged grips 128 preferably couples to the capture plate heater 123 and houses the capture plate module actuator 129, such that, in a first configuration, actuation of the capture plate module actuator 129 outwardly displaces the hinged grips (allowing the capture plate module 120 to receive a capture plate 110), and in a second configuration, actuation of the capture plate module actuator 129 inwardly displaces the

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hinged grips (allowing the capture plate module 120 to couple to the capture plate 110). The structural support with hinged grips 128 may further comprise a textured and/or high-friction surface configured to grip a capture plate 110, but alternatively may not comprise a textured and/or high-friction 5 surface.

The capture plate electronics module 127 is coupled to the capture plate heater 123 and the capture plate actuation system 126, and functions to enable control of the capture plate heater 123 and the capture plate actuation system 126. Preferably, the capture plate electronics module 127 modulates an output of the capture plate heater 123, in order to controllably heat at least one well 113 of the capture plate 110. Additionally, the capture plate electronics module 127 preferably modulates the capture plate actuation system 126, in order to 15 controllably couple the capture plate module 120 to a capture plate 110. Preferably, the capture plate electronics module 127 is coupled to an external power supply, such that the capture plate module 120 does not include an integrated power supply; however, in alternative embodiments, the cap- 20 ture plate electronics module 127 may be coupled to a power supply integrated with the capture plate module 120. 1.2 System—Molecular Diagnostic Module

As shown in FIGS. 6A and 6B, an embodiment of the molecular diagnostic module 130 of the system 100 includes 25 a cartridge receiving module 140, a heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, and an optical subsystem 180, and functions to manipulate a microfluidic cartridge 210 for processing of a biological sample containing nucleic acids. The molecular diagnostic 30 module 130 is preferably configured to operate in parallel with at least one other molecular diagnostic module 130, such that multiple microfluidic cartridges 210 containing biological samples may be processed simultaneously. In a first variation, the molecular diagnostic module 130 is configured to be 35 stackable with another molecular diagnostic module 130 in a manner that enables access to a microfluidic cartridge 210 within each molecular diagnostic module 130; an example of the first variation is shown in FIG. 6B, where the molecular diagnostic modules 130 are stacked in a staggered configu- 40 ration. In the first variation, each molecular diagnostic module 130 may further comprise locking pins or other appropriate mechanisms to couple the stacked molecular diagnostic modules 130 together. In another variation, the molecular diagnostic module 130 may not be configured to stack with 45 another molecular diagnostic module, such that the molecular diagnostic modules 130 are configured to rest side-by-side on the same plane. Elements of an embodiment of the molecular diagnostic module 130 are further described in sections 1.2.1 to 1.2.5 below.

1.2.1 Molecular Diagnostic Module—Cartridge Receiving Module

As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge platform 141 including a cartridge loading guiderail 142, a 55 cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141, and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol. As shown in FIGS. 7A-7C, the cartridge platform 141 is preferably configured to receive a microfluidic cartridge 210 along a cartridge loading 65 guiderail 142 until it reaches a cartridge stop 143, and be vertically displaced by the linear actuator 146, which places a

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biasing force against the set of springs 148 coupled to the cartridge platform 141. The magnet receiving slot 144 and the set of valve actuation slots 145 provide access, by a magnet 160 and a valve actuation subsystem 170, to the microfluidic cartridge 210, as the microfluidic cartridge is vertically displaced by the linear actuator 146.

The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment. Alternatively, the cartridge loading guiderails 142 and the cartridge stop 143 may be configured such that a microfluidic cartridge slides over or along the cartridge loading guiderails 142, after which the cartridge stop 143 couples to a portion of the microfluidic cartridge 210 to ensure proper alignment of the microfluidic cartridge. Additional variations of the cartridge loading guiderails 142 and the cartridge stop 143 may be used to enable reception and alignment of a microfluidic cartridge 210 by the molecular diagnostic module 130, and are known by those skilled in the art.

The embodiment of the cartridge platform 141 shown in FIG. 8 also includes a set of valve actuation slots 145, oriented perpendicular to the parallel cartridge loading guiderails 142 and configured to provide access to a valve actuation subsystem 170, and a magnet receiving slot 144 located among the set of valve actuation slots 145. Preferably, the magnet receiving slot 144 and the set of valve actuation slots 145 substantially span a long dimension of the cartridge platform 141, as shown in FIG. 8, and are configured to correspond to locations on a microfluidic cartridge 210 requiring a magnetic field and/or valving to enable processing of a biological sample and nucleic acid detection once the microfluidic cartridge 210 has been aligned within the molecular diagnostic module 130. Thus, alternative configurations of the magnet 50 receiving slot 144 and the set of valve actuation slots 145 may accommodate other cartridges with alternative regions requiring magnetic fields and/or valving to enable other protocols. In one alternative embodiment, the magnet receiving slot 144 and the set of valve actuation slots may comprise one continuous void of the cartridge platform 141, such that the cartridge platform 141 supports a microfluidic cartridge 210 along the periphery of the microfluidic cartridge 210, but forms a continuous void under a majority of the footprint of the microfluidic cartridge 210.

The linear actuator 146 functions to linearly displace a microfluidic cartridge 210 resting on the cartridge platform 141, in order to compress the microfluidic cartridge 210 and position the microfluidic cartridge 210 between a cartridge heater 153 and an optical subsystem 180 on one side of the microfluidic cartridge 210, and a magnet 160 and detection chamber heaters 157 on another side of the microfluidic cartridge 210. The linear actuator 146 also functions to provide

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a sufficient counterforce to the valve actuation subsystem 170 such that a microfluidic cartridge 210 within the molecular diagnostic module 130 remains properly situation upon manipulation by the valve actuation subsystem 170. The linear actuator 146 further functions to move a nozzle 149 5 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210. In the orientation of the molecular diagnostic module 130 shown in FIGS. 7B and 7B, the linear actuator **146** is preferably coupled to a portion of the heating and cooling subsystem 150 a portion of the optical subsystem 180, and the nozzle 149, and vertically displaces the cartridge heater 153, the optical subsystem 180, and the nozzle 149 to position the cartridge heater 153, 180 and the nozzle 149 over the microfluidic cartridge 210. The vertical displacement also 15 allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the 20 nucleic acid (e.g. PCR). Preferably, the linear actuator 146 is a scissor jack actuator configured to apply substantially uniform pressure over all occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and to operate in at least two configurations. In a 25 retracted configuration 146a, as shown in FIG. 9A, the scissor jack actuator has not linearly displaced the cartridge platform 141, and in an extended configuration 146b, as shown in FIG. 9B, the scissor jack actuator has linearly displaced the microfluidic cartridge 210 to position the microfluidic cartridge 210 30 between the subsystems 153, and 180, and the magnet 160 and detection chamber heaters 157. Additionally, the extended configuration 146b of the scissor jack actuator is configured to couple the nozzle 149 to a fluid port 222 of the microfluidic cartridge 210, such that the liquid handling sys- 35 tem 250 can deliver solutions and gases for processing of biological samples. The linear actuator 146 may alternatively be any appropriate linear actuator, such as a hydraulic, pneumatic, or motor-driven linear actuator, configured to linearly displace a microfluidic cartridge within the molecular diag- 40 nostic module 130.

As shown in FIGS. 7B, 7C, and 8, a set of springs 148 is coupled to the cartridge platform 141 and functions to provide a counteracting force against the linear actuator 146 as the linear actuator 146 displaces a microfluidic cartridge 210 45 resting on the cartridge platform 141. The set of springs 148 thus allows the cartridge platform 141 to return to a position that allows the microfluidic cartridge 210 to be loaded and unloaded from the molecular diagnostic module 130 when the linear actuator 146 is in a retracted configuration 146b, as 50 shown in FIG. 7B. Preferably, in the orientation shown in FIG. 7B, the set of springs 148 is located at peripheral regions of the bottom side of the cartridge platform 141, such that the set of springs 148 does not interfere with the magnet or the valve actuation subsystem 170. Alternatively, the set of 55 springs 148 may be located at any appropriate position to provide a counteracting force against the linear actuator 146. In a specific example shown in FIG. 6A, the set of springs 148 comprises four springs located near corners of the bottom side of the cartridge platform 141, but in other variations, the 60 set of springs 148 may comprise any appropriate number of springs. Each spring of the set of springs 148 is also preferably housed within a guide to prevent deviations from linear vertical motions (in the orientation shown in FIG. 7B); however, each spring in the set of springs 148 may alternatively 65 not be housed within a guide. In an alternative embodiment of the molecular diagnostic module 130, the set of springs 148

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may altogether be replaced by a second linear actuator configured to linearly displace a microfluidic cartridge 210, resting on the cartridge platform 141, in a direction opposite to the displacements enforced by the linear actuator 146.

Similarly, the nozzle 149, the heating and cooling subsystem 150, the cartridge heater 153, and the magnet 160 are preferably coupled to springs, such that springs are positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. Alternatively an elastomeric material is preferably positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. The springs and/or elastomeric material function to provide proper functioning and alignment of subsystems of the molecular diagnostic module 130 as the linear actuator 146 is extended or retracted, contributing to reliability and a reduction in stack up tolerance risk. The springs and/or elastomeric material further function to allow more pressure to be applied to occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and an appropriate pressure to be applied to elements 149, 150, 153 and 160 of the molecular diagnostic module 130. Thus, proper contact is maintained between elements 149, 150, 153, and 160, and a microfluidic cartridge 210 being manipulated by the molecular diagnostic module. These elements are described in further detail below.

1.2.2 Molecular Diagnostic Module—Heating/Cooling Subsystem and Magnet

The heating and cooling subsystem 150 of the molecular diagnostic module 130 comprises a cartridge heater 153, a fan 155, and a set of detection chamber heaters 157 and functions to controllably heat portions of a microfluidic cartridge 210 for processing of a biological sample containing nucleic acids according to a molecular diagnostic protocol. In the orientation of an embodiment of the molecular diagnostic module 130 shown in FIGS. 7A-7C, the cartridge heater 153 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140 and configured to span a central region of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, the fan 155 is located at a back wall of the cartridge receiving module 140, and the set of detection chamber heaters 157 is located inferior to a set of detection chambers 213 of the microfluidic cartridge 210. In alternative embodiments of the molecular diagnostic module 130, the heating and cooling subsystem 150 may have any appropriate alternative configuration that provides controlled heating and cooling to a microfluidic cartridge within the molecular diagnostic module 130.

The cartridge heater 153 functions to transfer heat to a heating region 224 of a microfluidic cartridge 210, for inducing a pH shift to release bound nucleic acids from magnetic beads within the heating region 224. The cartridge heater 153 is preferably a plate-shaped heater configured to transfer heat to the microfluidic cartridge 210 only from one side of the cartridge heater 153, such that heat flows through one face of the plate-shaped heater to the microfluidic cartridge 210. In a specific example, the cartridge heater 153 is a silicon wafer etched to be conductive and form a resistance heater. In the preferred variation, the cartridge heater 153 is either flip-chip bonded (i.e., soldered to back side of a circuit board), or wire bonded to a circuit board, and then coupled using linear bearings and springs to a plate coupled to the linear actuator 146. The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing. In another variation, heating through one face is accomplished using a plate-shaped resistance heater that has one exposed face and thermal insulation

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covering all other faces, and in yet another variation heating through one face is accomplished using a Peltier heater. In a variation of the cartridge heater 153 using a Peltier heater, the cartridge heater 153 comprises a thermoelectric material, and produces different temperatures on opposite faces of the cartridge heater 153 in response to a voltage difference placed across the thermoelectric material. Thus, when a current flows through the Peltier heater, one face of the Peltier heater lowers in temperature, and another face of the Peltier heater increases in temperature. Alternative variations of the cartridge heater 153 can be used to appropriately transfer heat to a heating region 224 of the microfluidic cartridge 210.

Preferably, the cartridge heater 153 is configured to linearly translate with the linear actuator 146 of the cartridge receiving module 140, in order to align with a heating region 15 224 spanning a central portion of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In one variation, the cartridge heater 153 is preferably fixed relative to the linear actuator 146 such that (in the orientation shown in FIGS. 7B-7C), the cartridge heater 153 can only move 20 vertically with the linear actuator. In an alternative variation, the cartridge heater 153 may additionally be configured to translate laterally with a horizontal plane (in the orientation shown in FIGS. 7B-7C), such that the cartridge heater 153 can translate in at least two perpendicular coordinate planes. In 25 this alternative variation, the cartridge heater 153 can be configured to sweep across a surface of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, or to translate in response to motion of the microfluidic cartridge 210, such that the position of the cartridge heater 30 153 relative to a heating region 224 of the microfluidic cartridge 210 is always fixed.

The fan 155 functions to modulate heat control within the molecular diagnostic module 130, by enabling heat transfer from warm objects within the molecular diagnostic module 35 130 to cooler air external to the molecular diagnostic module 130. In the orientation shown in FIG. 6A, the fan 155 is preferably located at a back face of the molecular diagnostic module 130, such heat within the molecular diagnostic module 130 is transferred out of the back face of the molecular 40 diagnostic module 130 to cooler air external to the molecular diagnostic module. In a specific embodiment, the molecular diagnostic module 130 comprises four fans 155 located at the back face of the molecular diagnostic module 130; however, in alternative embodiments the molecular diagnostic module 45 130 may comprise any appropriate number of fans located at any appropriate position of the molecular diagnostic module 130. In one variation, the fan 155 may be passive and driven solely by convection currents resulting from motion of hot air within the molecular diagnostic module to cooler air outside 50 of the molecular diagnostic module; however, in alternative variations, the fan 155 may be motor-driven and configured to actively cool internal components of the molecular diagnostic module 130 if molecular diagnostic module elements exceed a certain threshold temperature.

The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210. Each detection chamber heater in the set of detection chamber heaters 157 is preferably configured to heat one side of one 60 detection chamber in the set of detection chambers 213, and is preferably located such that the extended configuration 146b of the linear actuator 146 of the cartridge receiving module 140 puts a detection chamber in proximity to a detection chamber heater. As mentioned above, the set of detection 65 chamber heaters 157 is preferably coupled to springs or an elastomeric layer to ensure direct contact between the set of

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detection chamber heaters and a set of detection chambers, without compressively damaging the set of detection chamber heater 157. Preferably, each detection chamber heater is configured to contact a surface of a detection chamber in the extended configuration 146b of the linear actuator 146; however, each detection chamber heater may be further configured to couple to a detection chamber in the extended configuration 146b of the linear actuator 146. In a first variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a flexible printed circuit board, with a set of springs coupled to an opposite surface of the flexible printed circuit board, such that each spring in the set of springs aligns with a detection chamber heater. In the first variation, contact between each detection chamber heater and a detection chamber is thus maintained by a biasing force provided by an individual spring through the flexible printed circuit board. In a second variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a rigid printed circuit board, with a set of springs coupled to an opposite surface of the rigid printed circuit board. In the second variation, the set of springs thus function to collectively transfer a force through the rigid printed circuit board to maintain contact between the set of detection chamber heaters and a set of detection chambers. Preferably, each detection chamber heater in the set of detection chamber heaters 157 is configured to contact and heat a bottom surface of a detection chamber (in the orientation shown in FIG. 7B); however, each detection chamber heater may alternatively be configured to contact and heat both a top and a bottom surface of a detection chamber. Additionally, each detection chamber heater preferably corresponds to a specific detection chamber of the set of detection chambers 213 and functions to individually heat the specific detection chamber; however, alternatively, each detection chamber heater may be configured to heat multiple detection chambers in the set of detection chambers 213. Preferably, all detection chamber heaters in the set of detection chamber heaters 157 are identical; however, the set of detection chamber heaters 157 may alternatively not comprise identical detection chamber heaters.

In one variation, each detection chamber heater in the set of detection chamber heaters 157 comprises a donut-shaped heater, configured to encircle a surface of a detection chamber. The donut-shaped heater may further include a conducting mesh configured to allow detection through the heater while still allowing efficient heat transfer to the detection chamber. In an alternative variation, each detection chamber heater in the set of detection chamber heaters 157 may include a plate-shaped Peltier heater, similar to Peltier cartridge heater 153 described above. In this alternative variation, each detection chamber heater is thus configured to heat one side of a detection chamber through one face of the detection chamber heater. In one specific example, the molecular diagnostic module 130 comprises 12 diced silicon wafers with conductive channels flip chipped to 12 detection chambers, providing resistive heating to each of the 12 detection chambers. In another specific example, the molecular diagnostic module 130 comprises a 12 Peltier detection chamber heaters configured to heat 12 detection chambers of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In other alternative variations, each detection chamber heater may comprise any appropriate heater configured to individually heat a detection chamber.

The magnet 160 of the molecular diagnostic module 130 functions to provide a magnetic field for isolation and extraction of nucleic acids bound to magnetic beads within a microfluidic cartridge 210, aligned within the molecular

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diagnostic module 130. Preferably, the magnet 160 is fixed within the molecular diagnostic module 130, such that the extended configuration 146b of the linear actuator 146 allows the magnet 160 to pass through the magnet receiving slot 144 of the cartridge receiving module 140 and into a magnet 5 housing region 218 of the microfluidic cartridge 210. In an example, as shown in FIGS. 7A-7C, the magnet 160 is a rectangular prism-shaped magnet 160 fixed under the cartridge platform 141, and configured to pass through the cartridge platform 141, into a magnet housing region 218 located under the heating region 224 of the microfluidic cartridge 210. Preferably, the magnet 160 is one of two or three magnets lined up in parallel, such that each of the fluidic pathways of a microfluidic cartridge housing the magnets is exposed to two or three times as much magnetic flux, and two to threes 15 times as many opportunities to capture magnetic beads. Alternatively, the magnet 160 is a single magnet configured to expose a set of fluidic pathways to a magnetic field. Preferably, the magnet 160 or group of multiple magnets is coupled 130. Additionally, the magnet holder is preferably composed of an insulating material, such that the magnet holder does not interfere with proper functioning of the cartridge heater 153. Alternatively, the magnet holder may not be composed of an insulating material.

In one variation, the magnet 160 or group of multiple magnets comprises a permanent magnet, composed of a magnetized material (e.g., a ferromagnet) providing a substantially fixed magnetic field. In an alternative variation, the magnet 160 or group of multiple magnets comprises an elec- 30 tromagnet configured to provide a modifiable magnetic field, such that the intensity of the magnetic field can be adjusted, the polarity of the magnetic field can be reversed, and the magnetic field can be substantially removed upon removal of a current flowing within the electromagnet. Preferably, the 35 magnet 160 or group of magnets is also fixed relative to the molecular diagnostic module 130; however, the magnet 160 or group of magnets may alternatively be configured to translate vertically (in the orientation shown in FIG. 7B), such that the magnet 160 or group of magnets can extend into and 40 retract from the magnet receiving slot 144 of the cartridge platform 141 and the magnet housing region 218 of the microfluidic cartridge 210. Additionally, the magnet 160 or group of magnets preferably rides on linear bearings and springs (or an elastomeric material) to ensure proper contact 45 with a microfluidic cartridge in an extended configuration 146b of the linear actuator 146, in a manner that allows most of force from the linear actuator 146 to translate to full occlusion of a subset of the set of occlusion positions (i.e., without leakage).

Alternative configurations and/or compositions of the magnet 160 may also be appropriate in facilitating isolation and extraction of nucleic acids bound to magnetic beads within the microfluidic cartridge 210.

1.2.3 Molecular Diagnostic Module—Valve Actuation Sub- 55 system

As shown in FIGS. 10A-11C, the valve actuation subsystem 170 of the molecular diagnostic module 130 comprises a set of pins 172 configured to translate linearly within a pin housing 175, by sliding a cam card 177 laterally over the 60 pins 172. The valve actuation subsystem 170 functions to provide a biasing force to deform an object in contact with the set of pins 172. In a configuration wherein a microfluidic cartridge 210 is aligned within the molecular diagnostic module 130, the valve actuation subsystem 170 thus functions to 65 occlude a fluidic pathway 220 of the microfluidic cartridge 210 at a set of occlusion positions 226, to control flow of a

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biological sample containing nucleic acids, reagents and/or air through the microfluidic cartridge 210. In an embodiment of the molecular diagnostic module shown in FIGS. 7D-7E, the set of pins 172 and the pin housing are located directly under the microfluidic cartridge 210, such that the set of pins can access the microfluidic cartridge 210 through the valve actuation accommodating slots 145 of the cartridge platform 141. The cam card 177 in the embodiment is positioned under the set of pins and is coupled to a linear cam card actuator 178 configured to laterally displace the cam card 177 to vertically displace pins of the set of pins 172. Preferably, as shown in FIG. 11A, the cam card 177 rests on a low friction surface configured to facilitate lateral displacement of the cam card 177; however, the cam card 177 may alternatively rest on a bed of ball bearings to facilitate lateral displacement of the cam card 177, or may rest on any feature that allows the cam card 177 to be laterally displaced by the linear cam card actuator 178

The cam card 177, as shown in FIGS. 7D and 11A, includes to a magnet holder within the molecular diagnostic module 20 a set of hills 176 and valleys 179, and functions to transform linear motion in one plane to vertical motion in another plane. In one variation, the cam card 177 is coupled to a linear actuator and contacts the ends of pins in a set of pins 172, such that when a hill 176 of the cam card 177 passes under a pin, the pin is in a raised configuration 177a, and when a valley 179 of the cam card 177 passes under a pin, the pin is in a lowered configuration 177b. The hills 176 and valleys 179 of the cam card 177 are preferably in a set configuration, as shown in FIG. 11B, such that lateral motion of the cam card 177 to a set position raises a fixed subset of the set of pins 172. In this manner, lateral movement of the cam card 177 to different positions of a set of positions consistently raises different subsets of the set of pins 172 to occlude different portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172. Thus, portions of a fluidic pathway 220 may be selectively occluded and opened to facilitate processing of a biological sample according to any appropriate tissue, cellular, or molecular diagnostic assay protocol. In one variation, the cam card is configured to be laterally displaced in two coordinate directions within a plane (e.g., by x-y linear actuators), and in another variation, the cam card is configured to be laterally displaced in only one coordinate direction within a plane (e.g., by a single linear actuator). In a specific example, the hills 176 of the cam card 177 are raised 1 mm above the valleys 179 of the cam card 177, the hills 176 and valleys 179 each have a 2 mm wide plateau region, and a hill 176 region slopes down to a valley region 179 at a fixed angle over a 2 mm length. In the specific example, the cam card 177 is driven by a Firgelli linear actuator. Alternative variations may include any appropriate configurations and geometries of a cam card with hills 176 and valleys 179, driven by any appropriate actuator.

In alternative embodiments of the valve actuation subsystem 170, the cam card 177 may be a cam card wheel comprising a set of hills 176 and valleys 179 on a cylindrical surface, and configured to convert rotary motion to linear (i.e., vertical) motion of the set of pins 172. The cam card wheel may be configured to contact ends of pins in the set of pins 172, and may be coupled to a motor shaft and driven by a motor. In other alternative embodiments of the valve actuation subsystem 170, the cam card 177 may altogether be replaced by a set of cams, each configured to individually rotate about an axis. In these alternative embodiments, rotating subsets of the set of cams raises corresponding subsets of the set of pins, and occludes specific portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172.

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The set of pins 172 functions to selectively occlude portions of a fluidic pathway 220 of a microfluidic cartridge 210 at least at subsets of a set of occlusion positions 226. The pins of the set of pins 172 are preferably cylindrical and, in the orientation shown in FIG. 11A, configured to slide over a cam 5 card 177 and within a pin housing 175. Each pin in the set of pins 172 preferably also includes a first spring 173 that functions to provide a counteracting force to restore a pin to a lowered configuration 177b; however, each pin in the set of pins 172 may alternative not include a first spring 173, and 10 rely solely on gravity to return to a lowered configuration 177b. Preferably, as shown in FIG. 11C, each pin is also composed of two parts separated by a second spring, which functions to allow sufficient force to fully occlude a microfluidic channel but prevents forces from being generated that 15 could damage the pin, microfluidic cartridge and/or cam card. Each pin also preferably comprises a first region 171 configured to slide within the pin housing 175, and a second region 174 configured to exit the pin housing 175. The second region 174 is preferably of a smaller dimension than the first region 20 171, such that each pin is constrained by the pin housing 175 to be raised by a limited amount. Alternatively, the first region 171 and the second region 174 may have any appropriate configuration to facilitate raising and lowering of a pin by a fixed amount. In a specific example, the valve actuation sub- 25 system 170 comprises 12 sets of pins 172 configured to selectively occlude 12 fluidic pathways 212 of a microfluidic cartridge 210 aligned within the molecular diagnostic module; however, other embodiments may comprise any appropriate number of sets of pins 172.

In the orientation shown in FIG. 11A, each pin in the set of pins 172 preferably has a circular cross section and round ends, configured to facilitate sliding within a pin housing 175, sliding over a cam card 177 surface, and occlusion of a fluidic pathway 220. Alternatively, each pin may comprise any 35 appropriate cross-sectional geometry (e.g., rectangular) and/or end shape (e.g., flat or pointed) to facilitate occlusion of a fluidic pathway 220. Preferably, the surface of each pin in the set of pins 172 is composed of a low-friction material to facilitate sliding motions (i.e., over a cam card 177 or within 40 a pin housing 175); however, each pin may alternatively be coated with a lubricant configured to facilitate sliding motions.

The pin housing 175 functions to constrain and guide the motion of each pin in the set of pins 172, as the cam card 177 45 slides under the set of pins 172. Preferably, the pin housing 175 comprises a set of pin housing channels 169 configured to surround at least one pin in the set of pins 172. In one variation, each pin in the set of pins 172 is surrounded by an individual channel of the set of pin housing channels 169; 50 however, in another variation a channel of the set of pin housing channels 169 may be configured to surround multiple pins in the set of pins 172. In an example shown in FIGS. 7D-7E and 11A, the pin housing is located under the cartridge platform 141, such that the set of pin housing channels 169 is 55 aligned with the set of valve actuation accommodating slots 145, to provide access, by the set of pins 172, to a microfluidic cartridge 210 aligned on the cartridge platform 141. In the example, the pin housing 175 thus constrains the set of pins 172, such that each pin can only move linearly in a vertical 60 direction. Each pin housing channel preferably has a constricted region 168 configured to limit the motion of a pin within a pin channel; however, each pin housing channel may alternatively not include a constricted region. Preferably, surfaces of the pin housing 175 contacting the set of pins 172 are 65 composed of a low friction material to facilitate sliding of a pin within a pin housing channel; however, surfaces of the pin

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housing 175 contacting the set of pins 172 may alternatively be coated with a lubricant configured to facilitate sliding motions. Other variations of the pin housing 175 and the set of pins 172 may include no additional provisions to facilitate sliding of a pin within a pin housing channel.

1.2.4 Molecular Diagnostic Module—Optical Subsystem

As shown in FIGS. 12A-12D, the optical subsystem 180 of the molecular diagnostic module 130 comprises a set of light emitting diodes (LEDs) 181, a set of excitation filters 182 configured to transmit light from the set of LEDs 181, a set of dichroic mirrors 183 configured to reflect light from the set of excitation filters 182 toward a set of apertures 185 configured to transmit light toward a set of nucleic acid samples, a set of emission filters 186 configured to receive and transmit light emitted by the set of nucleic acid samples, and a set of photodetectors 187 configured to facilitate analysis of light received through the set of emission filters 186. The optical subsystem 180 may further comprise a set of lenses 184 configured to focus light onto the set of nucleic acid samples. The optical subsystem 180 thus functions to transmit light at excitation wavelengths toward a set of nucleic acid samples and to receive light at emission wavelengths from a set of nucleic acid samples. Preferably, the optical subsystem 180 is coupled to an optical subsystem actuator 188 configured to laterally displace and align the optical subsystem 180 relative to the set of nucleic acid samples, and is further coupled to a linear actuator 146 of the cartridge receiving module 140 to position the optical subsystem 180 closer to the set of nucleic acid samples. Alternatively, the optical subsystem 180 may not be coupled to a linear actuator 146 of the cartridge receiving module 140, and may only be configured to translate laterally in one direction. In a specific example, the optical subsystem 180 comprises a set of 12 apertures, a set of 12 lenses, a set of 12 dichroic mirrors, a set of 12 excitation fillers, a set of 12 LEDs, a set of 12 emission filters, and a set of 12 photodetectors. In the specific example, as shown in FIG. 7A-7E, the optical subsystem 180 is located within the molecular diagnostic module 130 and coupled to the linear actuator 146 of the cartridge receiving module 140, such that, in the extended configuration 146b of the linear actuator 146, the optical subsystem 180 can be positioned closer to a microfluidic cartridge 210 aligned within the molecular diagnostic module. Conversely in the specific example, the optical subsystem 180 is positioned away from the microfluidic cartridge 210 in the retracted configuration 146a of the linear actuator 146. In the specific example, the optical subsystem 180 is further coupled to an optical subsystem actuator 188 configured to laterally displace the optical subsystem 180 relative to the microfluidic cartridge 210, such that the optical subsystem 180 can be aligned with a set of detection chambers 213 of the microfluidic cartridge 210.

Preferably, the set of LEDs 181 are not all identical but rather chosen to efficiently produce a certain band of wavelengths of light, such that light from the set of LEDs 181 can be filtered to appropriate narrow wavelengths for analysis of nucleic acid samples. Alternatively, all LEDs in the set of LEDs 181 may be identical, and produce white light comprising all wavelengths of visible light that is filtered to produce the desired wavelength, in which case the LEDs may be stationary. Preferably, the set of LEDs 181 includes phosphor-based LEDs, but the set of LEDs 181 may alternatively include any LEDs configured to provide light of the desired range of wavelengths. The LEDs of the set of LEDs 181 are preferably configured to emit light of wavelengths corresponding to at least one of the set of excitation filters 182, the set of dichroic mirrors 183, and the set of emission filters 186.

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The set of excitation filters 182 is configured to align with the set of LEDs 181 in the optical subsystem 180, and functions to transmit light at excitation wavelengths toward the set of dichroic mirrors 183 of the optical subsystem 180. Preferably, the set of excitation filters 182 are not identical excitation filters, but rather chosen to transmit the different desired ranges of excitation wavelengths. Alternatively, all excitation filters of the set of excitation filters 182 are identical, and configured to transmit light having a fixed range of excitation wavelengths. In one variation, the set of excitation filters 182 includes band pass filters, configured to transmit light between two bounding wavelengths, in another variation, the set of excitation filters 182 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of excitation filters 182 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of excitation filters 182 is interchangeable, such that individual excitation filters may be interchanged to provide different excitation wavelengths of light; however, the set of excitation filters 182 may alterna- 20 tively be fixed, such that the optical subsystem 180 is only configured to transmit a fixed range of excitation wavelengths.

The set of dichroic mirrors 183 is configured to align with the set of excitation filters 182, and functions to receive and 25 reflect light from the set of excitation filters 182 toward the detection chamber, such that light having a range of excitation wavelengths may be focused, through a set of apertures, onto a set of nucleic acid samples. The set of dichroic mirrors 183 also functions to receive and transmit light from a set of 30 emission filters 186 toward a set of photodetectors 187, which is described in more detail below. All dichroic mirrors in the set of dichroic mirrors 183 are preferably identical in orientation relative to the set of excitation filters 182 and the set of emission filters 186, and configured to reflect and transmit the 35 appropriate wavelengths of light for the given LED. Alternatively, the set of dichroic mirrors 183 may include identical dichroic mirrors, with regard to orientation, light transmission, and light reflection. In a specific example, in the orientation shown in FIG. 12A, the set of excitation filters 182 is 40 oriented perpendicular to the set of emission filters 186, with the set of dichroic mirrors 183 bisecting an angle between two planes formed by the faces of the set of excitation filters 182 and the set of emission filters 186. In the specific example, light from the set of excitation filters is thus substantially 45 reflected at a 90° angle toward the set of apertures 185, and light from the set of emission filters 186 passes in a substantially straight direction through the set of dichroic mirrors 183 toward the set of photodetectors 187. Other variations of the set of dichroic mirrors 183 may include any configuration of 50 dichroic mirrors, excitation fillers, and/or emission filters that enable transmission of light of excitation wavelengths toward a set of nucleic acid samples, and transmission of light from the set of nucleic acid samples toward a set of photodetectors

In one embodiment, the optical subsystem may further include a set of lenses 184 configured to align with the set of dichroic mirrors 183, which functions to focus light, from the set of excitation filters 182 and reflected off of the set of figured to emit light in response to the light from the set of excitation filters 182. All lenses in the set of lenses 184 are preferably identical in orientation relative to the set of dichroic mirrors and in dimension; however, the set of lenses 184 may alternatively comprise non-identical lenses, such that 65 light passing through different lenses of the set of lenses 184 is focused differently on different nucleic acid samples. In a

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specific example, in the orientation shown in FIG. 12A, the faces of the set of lenses 184 are oriented perpendicular to the faces of the set of excitation filters 182, to account for light reflection by the set of dichroic mirrors 183 at a 90° angle. In the specific example, the set of lenses also includes identical 1/4" high numerical aperture lenses. In other variations, the set of lenses 184 may be oriented in any appropriate configuration for focusing light from the set of dichroic mirrors 183 onto a set of nucleic acid samples, and may include lenses of any appropriate specification (i.e., numerical aperture).

The set of apertures 185 is located on an aperture substrate 189 and configured to align with the set of lenses 184, and functions to allow focused light from the set of lenses 184 to pass through to the set of nucleic acid samples. The aperture substrate 189 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140, which allows the optical subsystem 180 to linearly translate and be positioned near and away from a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. Alternatively, the aperture substrate 189 may not be coupled to the linear actuator 146 of the cartridge receiving module 140. Preferably, all apertures 185 in the set of apertures 185 are identical, and configured to allow identical light profiles to be focused, through the set of lenses 184, onto a set of nucleic acid samples. Alternatively, the set of apertures 185 may not include identical apertures. In one variation, each aperture in the set of apertures 185 may be individually adjustable, in order to provide individually modifiable aperture dimensions (e.g., width, length, or diameter) to affect light exposure. In an alternative variation, each aperture in the set of apertures 185 is fixed. Other variations may include interchangeable aperture substrates 189, such that features of the set of apertures (e.g., aperture dimensions, number of apertures) may be adjusted by interchanging aperture substrates 189.

The set of emission filters 186 is configured to align with the set of dichroic mirrors, and functions to transmit emission wavelengths of light from the set of nucleic acid samples, and to filter out excitation wavelengths of light. Preferably, each emission filter of the set of emission filters 186 are configured to transmit light having a fixed range of emission wavelengths, while blocking light of excitation wavelengths. Alternatively, the set of emission filters 186 may comprise identical emission filters, such that individual emission filters of the set of emission filters 186 are configured to transmit the same ranges of emission wavelengths. In one variation, the set of emission filters 186 includes band pass fillers, configured to transmit light between two bounding wavelengths, in another variation, the set of emission filters 186 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of emission filters 186 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of emission filters 186 is interchangeable, such that individual emission filters may be interchanged to transmit and/or block different 55 wavelengths of light; however, the set of emission filters 186 may alternatively be fixed, such that the optical subsystem 180 is only configured to transmit a fixed range of emission wavelengths.

The set of photodetectors 187 is configured to align with dichroic mirrors 183, onto a set of nucleic acid samples con- 60 the set of emission filters 186, and functions to receive light from the set emission filters to facilitate analysis of the set of nucleic acid samples. All photodetectors in the set of photodetectors 187 are preferably identical; however, the set of photodetectors 187 may alternatively include non-identical photodetectors. Preferably, the set of photodetectors 187 includes photodiodes comprising a photoelectric material configured to convert electromagnetic energy into electrical

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signals; however, the set of photodetectors 187 may alternatively comprise any appropriate photodetectors for facilitating analysis of biological samples, as is known by those skilled in the art.

The optical subsystem actuator 188 is coupled to the opti- 5 cal subsystem 180, and functions to laterally translate the optical subsystem 180 relative to a set of nucleic acid samples being analyzed. Preferably, the optical subsystem actuator 188 is a linear actuator configured to translate the optical subsystem 180 in one dimension; however, the optical subsystem actuator 188 may alternatively be an actuator configured to translate the optical subsystem 180 in more than one dimension. In a specific example, as shown in FIGS. 7A-7D and 12D, the optical subsystem actuator 188 is configured to translate the optical subsystem 180 laterally in a horizontal 15 plane, to align the optical subsystem 180 with a set of detection chambers 213 of a microfluidic cartridge 210 within the molecular diagnostic module 130. In another example, the optical subsystem may be configured as a disc revolving around an axis with the LEDs and photodetectors stationary 20 and the disc containing the filters. In other variations, the optical subsystem actuator 188 may be configured in any appropriate manner to facilitate alignment of the optical subsystem 180 relative to a set of nucleic acid samples being analyzed.

1.2.5 Molecular Diagnostic Module—Alternative Embodiments and Variations

As described above, alternative embodiments of the molecular diagnostic module 130 and alternative variations of subsystems and elements of the molecular diagnostic module 130 may be configured to process a biological sample containing nucleic acids, isolate nucleic acids from the biological sample, and detect nucleic acids. An example of an alternative embodiment of a molecular diagnostic module 130, as shown in FIG. 13, includes a cartridge receiving 35 module 140', a heating and cooling subsystem 150', a magnet 160', a valve actuation subsystem 170', and an optical subsystem 180', and functions to manipulate an alternative microfluidic cartridge 210' for processing of biological samples containing nucleic acids. Other alternative embodi- 40 ments of the molecular diagnostic module 130" may be configured to receive alternative microfluidic cartridges 210", for processing of biological samples containing nucleic acids. 1.3 System—Assay Strip

As shown in FIGS. 18A and 18B, the assay strip 190 45 comprises an assay strip substrate 191 comprising a set of wells 192, and typically a puncturable foil seal 195, and functions to facilitate combination of a set of nucleic acid samples with a set of molecular diagnostic reagents for amplification and/or detection of a nucleic acid sequence or 50 sequences. Preferably, the entire assay strip 190 is configured to be a consumable (i.e., disposable), such that the assay strip 190 can be used during multiple runs of the system 100, then the assay strip 190 is disposed of once all of the wells 192, containing unitized reagents for a single test or group of tests, 55 is exhausted. Alternatively, at least a portion of the assay strip 190 is configured to be reusable, such that wells may be reloaded with reagents and reused with the system 100. In one variation of the assay strip 190, the assay strip substrate 191 is reusable, while the puncturable foil seal 195 is disposable and 60 replaced after each run of the system 100. In another variation, the reusable assay strip substrate 191 does not require a puncturable foil seal 195, such that reagents specific to a certain nucleic acid sequences may be deposited into open wells of the assay strip substrate 191 by a user.

The assay strip substrate 191 is configured such that the assay strip 190 is capable of resting on a flat surface, and

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functions to define the set of wells 192 and to couple to the puncturable foil seal 195. The assay strip substrate 191 is preferably configured to be received by a corresponding assay strip holder 230 configured to hold multiple assay strips 190, but may alternatively not be configured to couple to an assay strip holder 230. The assay strip substrate 191 is preferably composed of a PCR-compatible polymer, such as polypropylene, that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 192 of the assay strip substrate 191 function to receive at least one nucleic acid sample, and to facilitate combination of the nucleic acid sample with at least one of a set of molecular diagnostic reagents. The molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to perform alternative molecular diagnostic protocols. Preferably, the wells 193 of the assay strip substrate 191 are each configured to accommodate not only a nucleic acid sample, but also to facilitate mixing of the nucleic acid sample with at least one of a set of molecular diagnostic reagents (e.g., using a pipettor or other apparatus). Additionally, the molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprises probes and primers to detect the sample process controls provided by the capture plate, in order to verify process fidelity and assay accuracy. Preferably, the wells 193 are deep enough to facilitate mixing without splashing, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wide and shallow to facilitate drying of reagents in the wells to increase shelf life and larger devices for mixing the nucleic acids with molecular diagnostic reagents. Each well 193 of the set of wells 192 also preferably has a rounded bottom region, as shown in FIG. 18A, to facilitate complete aspiration of a fluid from a well 193; however, each well 193 may alternatively not have a rounded bottom region. Additionally, the set of wells 192 is preferably arranged in staggered rows, which functions to facilitate access to individual wells 193 of the set of wells, to reduce one dimension of the assay strip 190, and also to prevent cross-contamination of fluids within the wells due to dripping. Alternatively, the set of wells 192 may not be arranged in staggered rows.

The puncturable foil seal 195 functions to protect the molecular diagnostic reagents stored in wells 112 from degradation, isolate each well 193 of the set of wells 192, prevent contamination of the contents of each of the set of wells 192, and provide information identifying the assay strip 190. The puncturable foil seal 195 preferably seals each well 193 of the assay strip 190, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one variation, the puncturable foil seal 195 also forms a seal around an element that punctures it, and in another variation, the puncturable foil seal 195 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 195 is also preferably labeled with identifying information including at least one of manufacturer information, assay strip contents, the lot of the contents, an expiry date, and a unique

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electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal **195** does not extend beyond the footprint of the assay strip **190**, but alternatively, the puncturable foil seal **195** may be any appropriate size and/or include protruding features (e.g., tabs) that <sup>5</sup> facilitate handling of the assay strip.

In one variation, the assay strip 190 may be prepackaged with a set of molecular diagnostic reagents, such that each well 193 in the set of wells 192 is prepackaged with a quantity of molecular diagnostic reagents. The set of wells 192 may then be sealed by the puncturable foil seal 195, which is configured to be punctured by an external element that delivers volumes of nucleic acid samples to be combined with the set of molecular diagnostic reagents. In another variation, the assay strip 190 may not be prepackaged with a set of molecular diagnostic reagents, and the wells 193 of the assay strip 190 may not be sealed with a puncturable foil seal 195. In yet another variation, the system may comprise an empty assay strip 190 without a puncturable foil seal 195, and an assay 20 strip 190 comprising reagents and a puncturable foil seal 195, such that a user may add specific reagents to the empty assay strip to be used in conjunction with the assay strip comprising reagents. In variations comprising a puncturable foil seal 195, the puncturable foil seal 115 is configured to be punctured by 25 at least one external element, for co-delivery of nucleic acid samples and molecular diagnostic reagents intended to be combined.

In a specific example, the assay strip 190 has an 87 mm×16 mm footprint and comprises 24 wells 113 arranged in two 30 staggered rows, with a 9 mm center-to-center pitch between adjacent wells 193 within each row. Each well 193 of the set of wells has a capacity of 60 µL to accommodate a volume of a molecular diagnostic reagent, 20 µL of a sample fluid, and any displacement caused by a pipette tip (e.g., 100 or 300 μL 35 pipette tip). Each well 113 of the assay strip 190 in the specific example is also prepackaged with a quantity of molecular diagnostic reagents, and comprises a protruding top edge (75 microns high) that is heat sealed to a puncturable foil seal. The capture plate no in the specific example is produced by injec- 40 tion molding, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier. In the specific embodiment, the vapor barrier is further increased by depositing a thin metallic layer to the outside of the assay strip 190.

As described earlier, the assay strip 190 may be configured to be received by an assay strip holder 230. The assay strip holder 230 functions to receive and align multiple assay strips 190, such that a multichannel pipettor or other fluid delivery system may combine multiple nucleic acid samples with 50 molecular diagnostic reagents using wells 193 of multiple assay strips 190. In one variation, the assay strip holder 230 may be configured to contain Assay strips 190 including reagents for substantially different molecular diagnostic assays, as shown in FIG. 17B, such that a single run of the 55 system 100 involves analyzing a set of nucleic acid samples under different molecular diagnostic assays. In another variation, the assay strip holder 230 may be configured to contain assay strips 190 including reagents for identical molecular diagnostic assays, such that a single run of the system 100 involves analyzing a set of nucleic acid samples under the same molecular diagnostic assay. Preferably, the assay strip holder 230 is composed of a material that is dishwasher safe and autoclavable, configured to hold the assay strips 190 in place during handling by a fluid delivery system (e.g., pipettor), and configured such that the assay strips 190 avoid protruding over an edge of the assay strip holder 230, but the

assay strip holder 230 is constructed to facilitate insertion and removal of the assay strips 190 from the assay strip holder

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In one variation, the assay strip holder 230 is not configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190; however, in another variation as shown in FIG. 21A, the assay strip holder 230 may be further configured to couple to an aluminum block 235 coupled to a set of Peltier units 236 configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190. Additionally, the assay strip holder 230 may be configured to be received and carried by an assay strip carrier 240, which, as shown in FIG. 20, functions to facilitate handling and alignment of multiple assay strip holders 230. In a specific example, as shown in FIG. 19, the assay strip holder 230 has dimensions of 127.76 mm×85.48 mm×14.35 mm, complies with American National Standards Institute (ANSI) and Society for Laboratory Automation and Screening (SLAS) standards, and is configured to hold six 16-well assay strips for a total of 96 wells **193**. In another specific example, as shown in FIG. 21B, the assay strip holder 230' is configured to hold four assay strips 190', each comprising 24 wells 193' for a total of 96 wells per assay strip holder 230'. Other combinations of the described embodiments, variations, and examples of the assay strip 190, assay strip holder 230, and assay strip carrier 240 may be incorporated into embodiments of the system 100 for processing and detecting nucleic acids.

1.4 System—Microfluidic Cartridge

The microfluidic cartridge 210 functions to receive a set of magnetic bead-samples, facilitate separation of nucleic acids from the set of magnetic bead-samples, receive a set of nucleic acid-reagent samples, and facilitate analysis of nucleic acids from the set of nucleic acid-reagent samples. In one embodiment, the microfluidic cartridge 210 comprises a top layer 211 including a set of sample port-reagent port pairs 212 and a set of detection chambers 213; an intermediate substrate 214, coupled to the top layer 211 and partially separated from the top layer 211 by a film layer 215, configured to form a waste chamber 216; an elastomeric layer 217 partially situated on the intermediate substrate 214; a magnet housing region 218 accessible by a magnet 160 providing a magnetic field; and a set of fluidic pathways 219, each formed by at least a portion of the top layer 211, a portion of the film layer 215, and a portion of the elastomeric layer 217. In the embodiment, the microfluidic cartridge 10 further comprises a bottom layer 221 coupled to the intermediate substrate 214 and configured to seal the waste chamber 216. Furthermore, in the embodiment, the top layer 211 of the microfluidic cartridge 210 further comprises a shared fluid port 222, a vent region 223, and a heating region 224, such that each fluidic pathway 220 in the set of fluidic pathways 219 is fluidically coupled to a sample port-reagent port pair 224, the shared fluid port 222, the waste chamber 216, and a detection chamber 225, comprises a turnabout portion 226 configured to pass through the heating region 224 and the magnetic field, and is configured to pass through the vent region 223 upstream of the detection chamber 225. Each fluidic pathway 220 thus functions to receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 220.

The microfluidic cartridge 210 is preferably configured to be received and manipulated by the molecular diagnostic module 130, such that the cartridge receiving module 140 of the molecular diagnostic module 130 receives and aligns the microfluidic cartridge 210 within the molecular diagnostic module 130, the heating and cooling subsystem 150 of the molecular diagnostic module 130 is configured to transfer

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heat to the heating region 224 of the microfluidic cartridge 210, and the magnet 160 of the molecular diagnostic module 130 is configured to be received by the magnet housing region 218 of the microfluidic cartridge 210 to provide a magnetic field for separation of nucleic acids. Additionally, the shared fluid port 222 of the microfluidic cartridge 210 is configured to couple to a nozzle 149 coupled to the linear actuator 146 of the cartridge receiving module 140, such that the liquid handling system 250 can deliver fluids and gases through the shared fluid port 222. The elastomeric layer 217 of the microfluidic cartridge 210 is also preferably configured to be occluded at a set of occlusion positions 226 by the valve actuation subsystem 170 of the molecular diagnostic module, in order to occlude portions of a fluidic pathway 220 of the microfluidic cartridge 210 for processing of a set of biological samples. The optical subsystem 180 of the molecular diagnostic module 130 is further configured to align with the set of detection chambers 213 of the microfluidic cartridge 210, to facilitate analysis of a set of nucleic acid samples. The microf-20 luidic cartridge 210 is preferably the microfluidic cartridge 210 described in U.S. application Ser. No. 13/765,996, which is incorporated in its entirety by this reference, but may alternatively be any appropriate cartridge or substrate configured to receive and process a set of samples containing nucleic 25

#### 1.5 System—Fluid Handling System and Filter

The liquid handling system 250 of the system 100 includes a liquid handling arm 255 and a syringe pump 265, as shown in FIGS. 14A-14C and functions to deliver biological 30 samples, reagents, and gases to elements of the system 100. As described in Section 1, an embodiment of the liquid handling system 250 is configured to aspirate a set of biological samples containing nucleic acids (i.e., impure nucleic acid samples), dispense the set of biological samples into a capture 35 plate no to be lysed and combined with magnetic beads by a capture plate module 120, aspirate the set of biological samples combined with magnetic beads (i.e., set of magnetic bead-samples) from the capture plate 110, and dispense the set of magnetic bead-samples into microfluidic cartridge 210 40 located in a molecular diagnostic module 130. The embodiment of the liquid handling system 100 is further configured to facilitate separation of a set of nucleic acids from the magnetic bead-samples, by dispensing a wash solution, a release solution, and/or air into the molecular diagnostic 45 module 130, by the nozzle 149 coupled to the linear actuator 146, at appropriate stages, aspirate the set of nucleic acids from the molecular diagnostic module 130, combine the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, and dispense the set of nucleic acids 50 combined with the set of molecular diagnostic reagents (i.e., set of nucleic acid-reagent mixtures) into the molecular diagnostic module 130 for further processing and analysis. [Other embodiments of the liquid handling system 250 may be configured to perform alternative molecular diagnostic assay pro- 55 tocols and/or dispense and aspirate alternative fluids into and from other elements supporting a molecular diagnostic protocol.

The liquid handling arm 255 comprises a gantry 256 and a multichannel liquid handling head 257, and functions to 60 travel to different elements of the system 100 for fluid delivery and aspiration. The liquid handling arm 255 is preferably automated and configured to move, aspirate, and deliver fluids automatically, but may alternatively be a semi-automated liquid handling arm 255 configured to perform at least one of 65 moving, aspirating, and delivering automatically, while another entity, such as a user, performs the other functions.

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The gantry 256 is coupled to the multichannel liquid handling head 257, and functions to transport the multichannel liquid handling head 257 to different elements of the system 100 for fluid delivery and aspiration. Preferably, the gantry 256 is automated and configured to translate the multichannel liquid handling head 257 within at least two dimensions, and provides X-Y positional accuracy of at least 0.5 mm. Additionally, in the orientation shown in FIG. 14B, the gantry is preferably situated above the molecular diagnostic module 130, such that the gantry 256 can translate within at least two dimensions without interfering with other elements of the system 100. Alternatively, the gantry 256 may be any appropriate gantry 256 to facilitate movement of an end effector within at least two dimensions, as is readily known by those skilled in the art.

The multichannel liquid handling head 257 functions to aspirate fluids from and deliver fluids to different elements of the system 100. Preferably, the multichannel liquid handling head 257 is a multichannel pipette head; however, the multichannel liquid handling head 257 may alternatively be any appropriate multichannel liquid handling head configured to deliver fluids and/or gases. Preferably, the multichannel liquid handling head 257 comprises at least eight independent channels 258, but may alternatively comprise any number of channels 258 configured to aspirate and deliver fluids. The channel-to-channel pitch is preferably variable, and in a specific example ranges between 9 mm and 36 mm; however, the channel-to-channel pitch may alternatively be fixed, as shown in FIG. 15. The multichannel liquid handling head 257 also preferably provides independent z-axis control (in the orientation shown in FIG. 14B), such that, in combination with the gantry 256. The multichannel liquid handling head 257 is preferably configured to couple to both large (e.g., 1 mL) and small (e.g., between 100 and 300 µL) pipette tips, and in a specific example, has a precision of at least 6% using small disposable pipette tips and a precision of at least 2% using large disposable pipette tips when dispensing essentially the entire tip volume. Alternatively, the multichannel liquid handling head 257 may be configured to couple to any object configured to facilitate aspiration and delivery of fluids. Preferably, the multichannel liquid handling head 257 provides independent control of the channels 258, with regard to volumes of fluid aspirated or delivered, fluid dispensing rates, and/or engaging and disengaging pipette tips. Alternatively, the multichannel liquid handling head 257 may not provide independent control of the channels 258, such that all channels 258 of the multichannel liquid handling head 257 are configured to perform identical functions simultaneously. Preferably, the multichannel liquid handling head 257 is configured to aspirate and deliver both liquids and gases, but alternatively, the multichannel liquid handling head 257 may be configured to only aspirate and deliver liquids. Preferably, the multichannel liquid handling head 257 provides at least one of liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258; however, the multichannel liquid handling head 257 may alternatively not provide liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258.

In one embodiment, the multichannel liquid handling head 257 is configured to couple to at least one filter 260, which functions to pre-filter liquids being aspirated and/or dispensed by the liquid handling arm 255, and is preferably a custom filter 260 configured to couple to a pipette tip, but may alternatively be any appropriate filter configured to couple to the liquid handling arm 255 and filter liquids being aspirated and/or dispensed by the liquid handling arm 255.

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An embodiment of a custom filter 260, as shown in FIG. 22, comprises a first end 261 configured to couple to a pipette tip, a pointed second end 262, a void 263 coupled to the first end 261 and the pointed second end 262, and a filter membrane 264 subdividing the void 263. The first end 261, as shown in FIG. 22, preferably comprises a tapered channel configured to provide a friction fit with a pipette tip; however, the first end may alternatively not comprise a tapered channel and may be configured to couple to a pipette tip using any appropriate means. The pointed second end 262 is preferably sharp and configured to pierce an object, such as a foil seal; additionally, the pointed second end 262 is preferably at least as long as required to dispense into a well 113 of the capture plate 110. The void **263** preferably defines a conical region defined by the filter membrane 264, wherein the conical region is configured to divert a fluid within the filter 260 toward the pointed second end 262; however, the void 263 may not include a conical region. The filter membrane 264 functions to filter a fluid aspirated by the multichannel liquid handling head 257, 20 and is configured to subdivide the void 263 to define a conical region; however, the filter membrane 264 may alternatively not define a conical region of the void 263. In one embodiment, in the orientation shown in FIG. 22, the region of the void **263** below the filter membrane **264** may have a volumet- 25 ric capacity of between 200 ul and 1 mL; however, the region of the void 263 below the filter membrane may alternatively have any appropriate volumetric capacity.

A set of filters 260 may further be configured to be received and delivered by a filter holder 269, as, shown in FIG. 23. A 30 specific embodiment of a filter holder 269 comprises a set of 24 tapered holes with an 18 mm center-to-center pitch, arranged in six rows of four holes. The specific embodiment of the filter holder 269 is also compliant with ANSI and SLAS standards, has dimensions of 127.75×85.5×14.35 mm, and is 35 stackable with other specific embodiments of the custom filter holder 269. Alternatively, the filter holder 269 may be any appropriate filter holder 269 configured to receive and deliver a set of filters 260, as is readily known by those skilled in the art

#### 1.5.1 Fluid Handling System—Syringe Pump

The syringe pump 265 of the liquid handling system 250 is coupled to a wash solution source 266, a release solution source 267, a source of air 268, and flexible tubing 291, and functions to deliver a wash solution, a release solution, and air 45 through a valve to the molecular diagnostic module 130 to facilitate isolation and purification of nucleic acids from a set of magnetic bead-samples. The flexible tubing 291 is preferably coupled at a first end to the syringe pump, and at a second end to a nozzle 149 coupled to the linear actuator 146 of the 50 molecular diagnostic module 130, as shown in FIG. 14C. As stated earlier, an extended configuration 146b of the linear actuator 146 is configured to couple the nozzle 149 to a fluid port 222 of a microfluidic cartridge 210 within the molecular diagnostic module 130, such that the wash solution, release 55 solution, and air can be delivered to the microfluidic cartridge 210 at appropriate stages. A specific embodiment of the syringe pump 265 comprises a 4-way valve, is able to pump 20-5000 μL of fluids or air through the 4-way valve at flow rates from 50-500 µL/min, can couple to syringes with 60 between 1 mL and 10 mL capacities, and has a precision of at least 5% with regard to fluid or air delivery. Alternatively, the syringe pump 265 may be any appropriate syringe pump 265 or fluid delivery apparatus configured to deliver a wash solution, a release solution, and air to the molecular diagnostic 65 module 130, as is readily known by those skilled in the art. 1.6 System—Additional Elements

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The system 100 may further comprise a tag reader 271, which functions to read barcodes, QR codes and/or any other identifying tags of the system 100. Preferably, the tag reader 271 is coupled to the liquid handling system 250, such that the tag reader 271 is configured to read tags on puncturable foil seals 115, 195 or tags located on any element of the system 100 accessible by the liquid handling system 250; however, the tag reader 271 may alternatively not be coupled to the liquid handling system 250. In one alternative embodiment of the system 100, the tag reader 271 may be a standalone unit that is configured to be manipulated by a user to scan tags or labels located on elements of the system 100.

The system 100 may also further comprise a controller 272 coupled to at least one of the capture plate module 120, the molecular diagnostic module 130, the liquid handling system 250, and the tag reader 271, and functions to facilitate automation of the system 100. In a variation wherein the controller 272 is coupled to the capture plate module 120, the controller 272 preferably functions to automate heating of a capture plate 110, which facilitates lysing of biological samples within the capture plate 110 and binding of nucleic acids within the capture plate 110 to magnetic beads 119 of the capture plate 110. In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of nucleic acid-reagent mixtures by the optical subsystem 180. In a variation wherein the controller 272 is coupled to the liquid handling system 250, the controller 272 preferably functions to automate aspiration, transfer, and delivery of fluids and/or gases to different elements of the system 100. In a variation wherein the controller 272 is coupled to the tag reader 271, the controller preferably functions to automate reading of tags by the tag reader 271, and may further function to facilitate transfer of information from the tags to a processor 273. Other variations of a controller may function automate handling, 40 transfer, and/or storage of other elements of the system 100, such as capture plates 110, assay strips 190, assay strip holders 230, assay strip carriers 240, filters 200, filter holders 205, and/or microfluidic cartridges 210, using a robotic arm or gantry similar to that used in the liquid handling system 250. Alternative combinations of the above variations may involve a single controller 272, or multiple controllers configured to perform all or a subset of the functions described above.

The system 100 may also further comprise a processor 273, which functions to receive and process information from a tag reader 271, and also to receive and process data received from the optical subsystem 180 of the molecular diagnostic module 130. Preferably, the processor 273 is coupled to a user interface 274, which functions to display processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information. Alternatively, the processor 273 is not coupled to a user interface 274, but comprises a connection 275 configured to facilitate transfer of processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information to a device external to the system 100.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made the described embodiments of the system 100 without departing from the scope of the system 100.

2. Method for Processing and Detecting Nucleic Acids

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An embodiment of a method 400 for processing and detecting nucleic acids from a set of biological samples comprises: combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of 5 magnetic bead-sample mixtures S410; heating the set of magnetic bead-sample mixtures to produce a set of nucleic acidmagnetic bead samples S420; transferring each nucleic acidmagnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of 10 fluidic pathways S430; producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples S440; combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of 15 nucleic acid-reagent mixtures S450; transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers S460; and receiving light from the set of nucleic acid-reagent mixtures S470. 20 The method 400 may further comprise generating a set of data based on light received form the set of nucleic acid-reagent mixtures S480. The method 400 functions to isolate and extract a set of nucleic acid volumes from a biological sample, and to facilitate analysis of the nucleic acid volumes accord- 25 ing to at least one molecular diagnostic protocol.

Step S410 recites combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of magnetic bead-sample mixtures, and functions to prepare a set of biological samples to be lysed and 30 combined with magnetic beads. For each biological sample, Step S410 preferably comprises aspirating a portion of the volume of the biological sample from a sample container (possibly containing an aqueous solution prior to addition of biological sample), and transferring the portion of the bio- 35 logical sample to a well containing a set of magnetic beads. Alternatively, for each biological sample, Step S410 may comprise aspirating the entire volume of the biological sample from a sample container, and transferring the volume netic beads. Preferably, all biological samples in the set of biological samples are aspirated and combined with the magnetic beads in the wells simultaneously using a multichannel fluid delivery system; however, all biological samples in the set of biological samples may alternatively be aspirated and 45 combined with a set of magnetic beads non-simultaneously. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. 50 However, the magnetic beads may alternatively be any appropriate magnetic beads configured to facilitate biomagnetic

In addition to combination with magnetic beads, Step 410 may further include combining each biological sample of the 55 set of biological samples with a lysing enzyme (e.g. proteinase K), and a sample process control comprising two or more nucleic acid sequences (i.e., one for DNA and one for RNA) to be included with each sample. This allows biological samples to effectively lysed, which releases waste compo- 60 nents into a wash solution, and allows nucleic acids to bind to magnetic beads. This additionally allows the sample process control to be later detected, as a check to verify the accuracy of a molecular diagnostic assay being performed.

In a first variation of Step S410 for one biological sample, 65 as shown in FIG. 16A, a volume of the biological sample is aspirated and combined with a set of magnetic beads. In the

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first variation of Step S410, a set of different biological samples may thus be aspirated simultaneously, and each biological sample may be transferred to an individual well to be combined with a set of magnetic beads to produce a set of magnetic bead-sample mixtures. In the first variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic bead-sample mixtures are substantially non-identical in composition. In a second variation of Step S410, as shown in FIG. **16**B, a volume of a stock biological sample is aspirated, and portions of the volume of the stock biological sample are transferred to multiple wells to be combined with multiple sets of magnetic beads to produce a set of magnetic beadsample mixtures. In the second variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic beadsample mixtures are substantially identical in composition. Other variations of Step S410 may comprise filtering at least one biological sample of the set of biological samples S415 prior to combining each biological sample of the set of biological samples with a quantity of magnetic beads.

In a specific example of Step S410, a multichannel liquid handling system aspirates approximately 1 mL of each of a set of biological samples in aqueous buffer using a set of 1 mL pipette tips, couples each of the pipette tips to a custom 13 mm diameter filter, punctures a foil seal 115 of a capture plate at a set of wells, wherein each well of the set of wells contains a set of magnetic beads, and dispenses each aspirated volume of a biological sample into a well of the capture plate containing a set of magnetic beads, and disposes of the tip/filter combination. In the specific example of Step S410, the multichannel liquid handling system then picks up new disposable tips and aspirates and dispenses the contents of each well at least three times to mix the contents, and then disposes of the set of pipette tips and filters.

Step S420 recites heating the set of magnetic bead-sample mixtures to produce a set of nucleic acid-magnetic bead samples, and functions to incubate the set of magnetic beadsample mixtures in order to lyse biological matter, and release nucleic acids to be bound to magnetic beads. Preferably, Step S420 comprises heating a capture plate containing the set of of the biological sample to be combined with a set of mag- 40 magnetic bead-sample mixtures for a specified amount of time at a specified temperature, and may additionally include cooling the set of magnetic bead-sample mixtures. In a specific example, Step S420 comprises heating a capture plate containing the set of magnetic bead-sample mixtures using a capture plate module, wherein the capture plate module is configured to cradle and controllably heat wells containing the set of magnetic bead-sample mixtures. Step S420 may alternatively comprise incubating the set of magnetic beadsample mixtures using any appropriate method and/or system as is known by those skilled in the art. Finally, Step S420 may be omitted in embodiments of the method 400 involving samples that do not require heating.

Step S430 recites transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways, and functions to isolate each of the set of nucleic acid-magnetic bead samples within separate pathways for further processing. Preferably, all nucleic acid-magnetic bead samples in the set of nucleic acid-magnetic bead samples are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-magnetic bead sample in the set of magnetic bead-samples may be transferred to a corresponding fluidic pathway independently of the other nucleic acid-magnetic bead samples. In addition, preferably the entire volume, or substantially all of the volume, of the nucleic acid-magnetic bead sample is transferred to the set of fluidic pathways, without magnetically isolating magnetic

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beads and removing supernatant fluids prior to transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways.

Step S430 may further comprise occluding at least one 5 fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S432, which functions to define at least one truncated fluidic pathway. Preferably, Step S432 comprises defining at least one truncated fluidic pathway passing through at least one of a heating region and a magnetic field; however, Step S432 may alternatively not comprise defining a truncated fluidic pathway passing through at least one of a heating region and a magnetic field.

In a specific example of Step S430, the multichannel liquid handling subsystem of Step S410 transfers a set of nucleic 15 acid-magnetic bead samples to a set of fluidic pathways of a microfluidic cartridge aligned within a molecular diagnostic module, wherein the microfluidic cartridge comprises an elastomeric layer in contact with the set of fluidic pathways. Manipulation of the elastomeric layer at a subset of a set of 20 occlusion positions by a valve actuation subsystem of the molecular diagnostic module defines a set of truncated fluidic pathways crossing a heating region and a magnetic field, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a trun- 25 cated fluidic pathway of the set of truncated fluidic pathways.

Step S440 recites producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples, and functions to separate nucleic acid volumes from the set of nucleic acid-magnetic bead samples. Step S440 preferably 30 reduces a concentration of unwanted matter from the set of biological samples being processed, to an acceptable level; however, Step S440 may alternatively entirely remove substantially all unwanted substances from the set of biological samples being processed. Step S440 preferably includes pro- 35 viding a magnetic field S441, such that each fluidic pathway in the set of fluidic pathways is configured to cross the magnetic field. Preferably, the set of nucleic acid-magnetic bead samples is captured and isolated within portions of the set of fluidic pathways crossing the magnetic field. Step S440 may 40 further comprise providing a heater configured to span a heating region of the set of fluidic pathways S442, but may alternatively comprise providing multiple heaters or altogether omit providing a heater. In embodiments wherein multiple heaters are provided, each heater is preferably indepen- 45 dent to allow independent control of heating time and temperature for each sample. Step S442 functions to provide a heater, which, in combination with a release solution that provides a pH shift, facilitate a rapid and efficient unbinding of the nucleic acids from magnetic beads.

Step S440 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S443 (and opening a previously occluded channel), which functions to define at least one bead sample and coupled to a source for delivery of a wash solution and a release solution. Preferably, Step S443 comprises defining at least one truncated fluidic pathway coupled to a waste chamber and to a fluid port, which functions to facilitate washing of at least one nucleic acid-magnetic bead 60 sample in the set of nucleic acid-magnetic bead samples, and releasing of at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. Step S440 may additionally comprise delivering a wash solution through a portion of at least one fluidic pathway S444, such as the truncated 65 fluidic pathway defined in Step S443, and delivering a release solution through a portion of at least one fluidic pathway

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S445, such as the truncated fluidic pathway defined in Step S443. Step S444 functions to wash at least one nucleic acidmagnetic bead sample in the set of nucleic acid-magnetic bead samples, and Step S445 functions to release at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. The heater provided in Step S442 may be activated after Step S445 to induce a pH shift.

In a specific example of Step S440, the set of fluidic pathways containing a set of nucleic acid-magnetic bead samples, from the specific example of Step S430, is occluded at a subset of the set of occlusion positions by a valve actuation subsystem of the molecular diagnostic module, to define a set of truncated fluidic pathways coupled to a waste chamber and to a shared fluid port of the microfluidic cartridge for delivery of a wash solution and a release solution. The liquid handling system delivers a wash fluid through the shared fluid port to wash the set of nucleic acid-magnetic bead samples, captured within the magnetic field, and then delivers a release fluid through the shared fluid port to release a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples. In the specific example, each fluidic pathway is washed sequentially, and the release solution is delivered to each fluidic pathway sequentially to ensure that each lane is provided with substantially equal amounts of wash and release solutions. All waste fluid produced in the specific example of Step S440 pass into the waste chamber coupled to the set of truncated fluidic pathways.

Step S450 recites combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures, which functions to prepare the set of nucleic acid volumes to be detected. For each nucleic acid volume in the set of nucleic acid volumes, Step S450 preferably comprises aspirating an entire volume of the nucleic acid volume from its corresponding fluidic pathway, and transferring the nucleic acid volume to a well containing a molecular diagnostic reagent. Preferably, all nucleic acid volumes in the set of nucleic acid volumes are aspirated and combined with molecular diagnostic reagents simultaneously using a multichannel fluid delivery system; however, each nucleic acid volume in the set of nucleic acid volumes may alternatively be aspirated and combined with molecular diagnostic reagents independently of the other nucleic acid volumes. The molecular diagnostic reagents preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to detect any specific nucleic acid

In a first variation of Step S450 as shown in FIG. 16A, a truncated fluidic pathway containing a nucleic acid-magnet 55 nucleic acid volume is aspirated and combined with a molecular diagnostic reagent for a single assay. In the first variation of Step S450, a set of nucleic acid volumes may thus be aspirated simultaneously, and each nucleic acid volume may be transferred to an individual well to be combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures. In the first variation of Step S450, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures may or may not be substantially identical in composition, depending on the homogeneity of the biological samples used in Step S410; however, the first variation of S450 preferably comprises using identical molecular diagnostic reagents, such

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that identical molecular diagnostic protocols analyzing identical markers may be performed. Thus, the first variation of Step S450 encompasses running multiple identical tests from a stock biological sample (e.g., a multiplex assay), and running identical tests using a set of substantially different biological samples (e.g., from different sources).

In a second variation of Step S450, as shown in FIG. 16B, the set of nucleic acid volumes is aspirated, and each nucleic acid volume in the set of nucleic acid volumes is combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents of Step S450, the set of molecular diagnostic reagents preferably comprises different molecular diagnostic reagents, such that different molecular diagnostic protocols analyzing different markers may be performed. Thus, the second variation encompasses running multiple substantially different tests using a stock biological sample, and running substantially different tests using substantially different biological samples (e.g., from different sources)

In a specific example of Step S450, a multichannel liquid 20 handling system aspirates approximately  $18~\mu L$  of each of a set of nucleic acid volumes from the microfluidic cartridge used in the specific example of Step S440 using a set of pipette tips, punctures at least one foil seal 195 of at least one assay strip, wherein each well of the at least one assay strip contains 25 molecular diagnostic reagents, and dispenses each aspirated nucleic acid volume into a well of the assay strip. In the specific example of S450, the multichannel liquid handling system then aspirates and dispenses the contents of each well approximately 10 times to reconstitute molecular diagnostic 30 reagents and mix the contents of each well.

Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the 35 set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent 40 mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures. Step S460 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion posi- 45 tions S462, which functions to define at least one truncated fluidic pathway coupled to a detection chamber of a set of detection chambers. Preferably, Step S462 comprises occluding each fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions, thus defining a set of 50 truncated fluidic pathways, each coupled to a detection cham-

In a specific example of Step S460, the multichannel liquid handling subsystem of the specific example of Step S450 transfers a set of nucleic acid-reagent mixtures, each having a 55 volume of approximately 16  $\mu L$ , back to the set of fluidic pathways of the microfluidic cartridge of the specific example of Step S450. Each nucleic acid-reagent mixture in the set of nucleic acid-reagent mixtures is transferred at a rate of 50  $\mu L/\text{minute}$ . Manipulation of the elastomeric layer at a subset of a set of occlusion positions by the valve actuation subsystem of the molecular diagnostic module defines a set of truncated fluidic pathways, each coupled to a detection chamber, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a 65 truncated fluidic pathway of the set of truncated fluidic pathways. In the specific embodiment the occlusion position

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immediately upstream of the detection chamber and the occlusion position immediately downstream of the detection chamber are normally closed positions. During delivery, the multichannel liquid handling subsystem generates pressure to cause the elastomeric layer at the normally closed positions to deform and allow fluid to flow through the normally closed positions. Once the pressure drops after the detection chamber is filled and the multichannel liquid handing subsystem ceases delivery, the elastomeric layer is configured to overcome the pressure in the channel and recloses, thereby sealing the normally closed positions. The normally closed positions are then compressed using the valve actuation subsystem during thermocycling to prevent pressures generated during a molecular diagnostic assay to cause the normally closed positions to leak. After the molecular diagnostic assay is complete and the occlusion "pins" withdrawn, the normally closed positions allow the samples and amplicons to be trapped within detection chambers, substantially reducing the risk of contamination of the lab or other samples.

Step S470 recites receiving light from the set of nucleic acid-reagent mixtures, and functions to produce emission responses from the set of nucleic acid-reagent mixtures in response to transmission of excitation wavelength light or chemiluminescent effects. Preferably, Step S470 comprises the ability to transmit light including a wide range of wavelengths through a set of excitation filters and through a set of apertures configured to individually transmit light having single or multiple excitation wavelengths onto the set of nucleic acid-reagent mixtures, and receiving light through a set of emission filters, from the set of nucleic acid-reagent mixtures. Step S470 may additionally comprise reflecting light from the set of excitation filters off of a set of dichroic mirrors, and transmitting light through the set of dichroic mirrors to a set of photodetectors. A specific example of Step S470 comprises using the optical subsystem 180 of the system 100 described above to transmit and receive light; however, alternative variations of Step S470 may use any appropriate optical system configured to transmit light at excitation wavelengths toward the set of nucleic acid-reagent mixtures, and to receive light at emission wavelengths from the set of nucleic acid-reagent mixtures.

Step S480 recites generating a set of data based on light received from the set of nucleic acid-reagent mixtures, which functions to produce quantitative and/or qualitative data from the set of nucleic acid-reagent mixtures. Step S480 may further function to enable detection of a specific nucleic acid sequence from the nucleic acid-reagent mixture, in order to identify a specific nucleic acid sequence, gene, or organism. Preferably, Step S480 includes converting electrical signals, produced by a set of photodetectors upon receiving light from the set of nucleic acid-reagent mixtures, into a quantifiable metric; however, S480 may alternatively comprise converting electromagnetic energy, received by a set of photodetectors from the set of nucleic acid-reagent mixtures, into a set of qualitative data. In one variation of Step S480, the set of data may be processed by a processor and rendered on a user interface; however, in other variations of Step S480, the set of data may alternatively not be rendered on a user interface.

The method 400 may further comprise re-running a biological sample S490 if processing and/or analysis of the biological sample results in less than ideal results. Preferably, Step S490 occurs if an analysis of a biological sample is indeterminate due to machine or user error. Additionally, Step S490 preferably occurs automatically upon detection of a less than ideal result, but may alternatively occur in response to a user prompt

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Embodiments of the method **400** and variations thereof can be embodied and/or implemented at least in part by a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system **100** and one or more portions of the processor **273** and/or the controller **272**. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instructions.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of systems, methods and computer program products according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block 20 diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted 25 in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart 30 illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

We claim:

- 1. A system for processing and detecting nucleic acids, comprising:
  - a capture plate comprising at least one well configured to facilitate a combination of a set of magnetic beads with 45 a biological sample, thus producing a magnetic beadsample; and
  - a molecular diagnostic module, configured to process at least one magnetic bead-sample obtained from the capture plate, and separate nucleic acids from magnetic 50 beads, wherein the molecular diagnostic module comprises:
    - a cartridge platform comprising a magnet receiving slot, an actuator configured to displace the cartridge platform.
    - a magnet, wherein an extended configuration of the actuator allows the magnet to pass through the magnet receiving slot to facilitate separation of the at least one nucleic acid volume, and
  - a cam card contacting a set of pins, wherein the extended 60 configuration of the actuator combined with movement of the cam card displaces a subset of the set of pins through a set of slots of the cartridge platform, to define at least one distinct pathway configured to receive at least one magnetic bead-sample.
- 2. The system of claim 1, further comprising a liquid handling system configured to transfer at least one magnetic

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bead-sample from the capture plate to the molecular diagnostic module, and to aspirate at least one nucleic acid volume from the molecular diagnostic module.

- 3. The system of claim 2, wherein the system is not configured to remove a supernatant from the capture plate prior to transfer of at least one magnetic bead-sample from the capture plate to the molecular diagnostic module.
- 4. The system of claim 1, wherein the capture plate comprises a set of wells and a foil seal configured to seal each well of the set of wells, wherein each well in the set of wells contain a set of magnetic beads, a set of lysing reagents, and a sample process control.
- 5. The system of claim 1, further comprising a capture plate module comprising a thermally conducting substrate configured to cradle at least one well of the capture plate, and a heater coupled to the thermally conducting substrate.
  - **6**. The system of claim **1**, wherein the actuator of the molecular diagnostic module is a linear actuator, configured to vertically displace the cartridge platform, and wherein the cartridge platform of the molecular diagnostic module is coupled to a set of springs configured to counteract a force provided by the linear actuator.
  - 7. The system of claim 1, wherein a retracted configuration of the actuator allows the magnet to retract from the magnet receiving slot.
  - 8. The system of claim 1, wherein the magnet is configured to provide a magnetic field spanning at least three fluidic pathways configured to facilitate isolation and extraction of three nucleic acid volumes.
  - 9. The system of claim 1, wherein the magnet is at least one of an electromagnet and a permanent magnet.
- 10. The system of claim 1, further comprising an assay strip comprising at least one reagent well, each containing a molecular diagnostic reagent configured to be combined with a nucleic acid volume.
- 11. The system of claim 9, wherein the molecular diagnostic module further comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward one of three nucleic acid-reagent mixtures, and to transmit emitted light from one of the three nucleic acid-reagent mixtures, through the emission filter, and toward the photodetector.
  - 12. The system of claim 1, wherein the molecular diagnostic module further comprises a molecular diagnostic module heater configured to heat at least one magnetic bead-sample.
  - 13. The system of claim 1, wherein the molecular diagnostic module is configured receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.
  - **14**. The system of claim **1**, wherein the liquid handling system comprises a multichannel pipette head and a syringe pump.
- 15. The system of claim 14, wherein the syringe pump is coupled to a nozzle coupled to the actuator of the moleculardiagnostic module.
  - 16. A system for processing and detecting nucleic acids, comprising:

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- a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample to produce a magnetic bead-sample;
- an assay strip comprising at least one well containing a molecular diagnostic reagent configured to be combined 5 with a nucleic acid volume to produce a nucleic acidreagent mixture;
- a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate, separate the nucleic acid volume from the magnetic bead-sample, 10 and analyze the nucleic acid-reagent mixture from the assay strip,
  - wherein the molecular diagnostic module comprises a cartridge platform including a set of parallel slots, a cam card, and a set of pins contacting the cam card, 15 wherein movement of the cam card displaces a subset of the set of pins through a subset of the set of parallel slots to define at least one pathway configured to receive the magnetic bead-sample; and
- a liquid handling system configured to transfer the mag- 20 netic bead-sample from the capture plate to the molecular diagnostic module, transfer the nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer the nucleic acid-reagent mixture from the assay strip to the molecular diagnostic module.
- 17. The system of claim 16, wherein the capture plate comprises a first foil seal configured to seal each well of the set of wells, and wherein the assay strip comprises a second foil seal, configured to seal each reagent well of the set of reagent wells.
- 18. The system of claim 16, wherein the molecular diagnostic module comprises an optical subsystem comprising at least one unit, wherein each unit includes an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the  $\,^{35}$ excitation filter toward the nucleic acid-reagent mixture, and to transmit light from the nucleic acid reagent mixture. through the emission filter, and toward the photodetector wherein each unit of the optical subsystem further comprises an LED aligned with the excitation filter, wherein the LED 40 provides multiple wavelengths of light corresponding to at least one of the excitation filter, the dichroic mirror, and the emission filter.
- 19. The system of claim 16, wherein the molecular diagnostic module further comprises a heater and a detection chamber heater, wherein the heater is configured to heat the magnetic bead-sample, and wherein the detection chamber heater is configured to individually heat the nucleic acidreagent mixture, and wherein at least one of the heater and the detection chamber heater is a Peltier heater.
- 20. The system of claim 18, wherein the molecular diagnostic module further comprises a linear actuator coupled to the optical subsystem, such that an extended configuration of the linear actuator vertically displaces the optical subsystem.
- system comprises a multichannel pipette head and a syringe
- 22. The system of claim 21, wherein the syringe pump is coupled to a nozzle coupled to the actuator of the molecular diagnostic module.
- 23. A system for processing and detecting nucleic acids, comprising:

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- a molecular diagnostic module, configured to process a set of biological samples combined with magnetic beads and separate a set of nucleic acid volumes from the set of biological samples combined with magnetic beads, wherein the molecular diagnostic module comprises an actuator configured to displace a cartridge, a set of pins, and at least one cam contacting the set of pins, wherein an extension or retraction of the actuator moves the cartridge, and a displacement of a subset of the set of pins defines a set of pathways in the cartridge, each configured to receive a biological sample combined with magnetic beads of the set of biological samples combined with magnetic beads.
- 24. The system of claim 23, further comprising:
- a capture plate comprising at least one well containing a set of magnetic beads configured to be combined with a biological sample, a lysing reagent, and a sample process control, wherein the capture plate facilitates production of at least one biological sample combined with magnetic beads;
- an assay strip comprising at least one reagent well containing a molecular diagnostic reagent volume configured to be combined with a nucleic acid volume of the set of nucleic acid volumes to produce at least one nucleic acid-reagent mixture; and
- a liquid handling system configured to transfer at least one biological sample combined with magnetic beads from the capture plate to the molecular diagnostic module, transfer at least one nucleic acid volume from the molecular diagnostic module to the assay strip, and transfer at least one nucleic acid-reagent mixture from the assay strip back to the molecular diagnostic module.
- 25. The system of claim 23, wherein the actuator of the molecular diagnostic module is a linear actuator, configured to vertically displace the cartridge.
  - 26. The system of claim 23, wherein the cartridge of the molecular diagnostic module contacts a cartridge platform coupled to a set of springs configured to counteract a force provided by the linear actuator.
- 27. The system of claim 26, further comprising a pin housing configured to house the set of pins and to guide the set of pins through a set of slots of the cartridge platform.
- 28. The system of claim 27, wherein at least one pin in the set of pins has a first diameter and a second diameter, different from the first diameter, such that the first diameter and the second diameter are configured to limit motion of the at least one pin through the pin housing.
- 29. The system of claim 28, wherein at least one pin in the set of pins includes a spring configured to counteract a force 50 provided by the cam.
  - 30. The system of claim 23, wherein the cam is a cam card is coupled to a cam card actuator configured to linearly displace the cam card under the set of pins.
- 31. The system of claim 30, wherein the cam card com-21. The system of claim 16, wherein the liquid handling 55 prises a fixed set of hills and valleys, such that a pin of the set of pins is raised when a hill of the cam card passes under the pin, and a pin of the set of pins is lowered when a valley of the cam card passes under the pin.
  - 32. The system of claim 24, wherein the liquid handling system comprises a multichannel pipette head and a syringe pump.

# EXHIBIT 25

US009101930B2

## (12) United States Patent Williams et al.

## (10) Patent No.: US 9,101,930 B2

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#### (54) MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC

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U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-

claimer.

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(51) Int. Cl.

B01L 3/00 (2006.01)

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(52) U.S. Cl.

CPC ........ B01L 3/5027 (2013.01); B01L 3/502707 (2013.01); B01L 3/502738 (2013.01); B01L 7/52 (2013.01); B01L 7/525 (2013.01); C12M 23/42 (2013.01); C12Q 1/68 (2013.01); C12Q 1/686 (2013.01); B01L 3/502 (2013.01); B01L 3/5025 (2013.01); B01L 3/50273 (2013.01);

B01L 3/502723 (2013.01); B01L 2200/0684 (2013.01); B01L 2200/0689 (2013.01); B01L 2200/10 (2013.01); B01L 2200/142 (2013.01); B01L 2300/087 (2013.01); B01L 2300/0809 (2013.01);

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(58) Field of Classification Search

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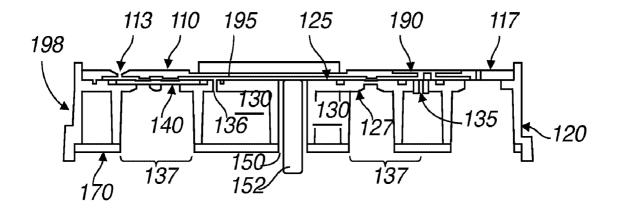
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#### (57) ABSTRACT

A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.

#### 28 Claims, 18 Drawing Sheets

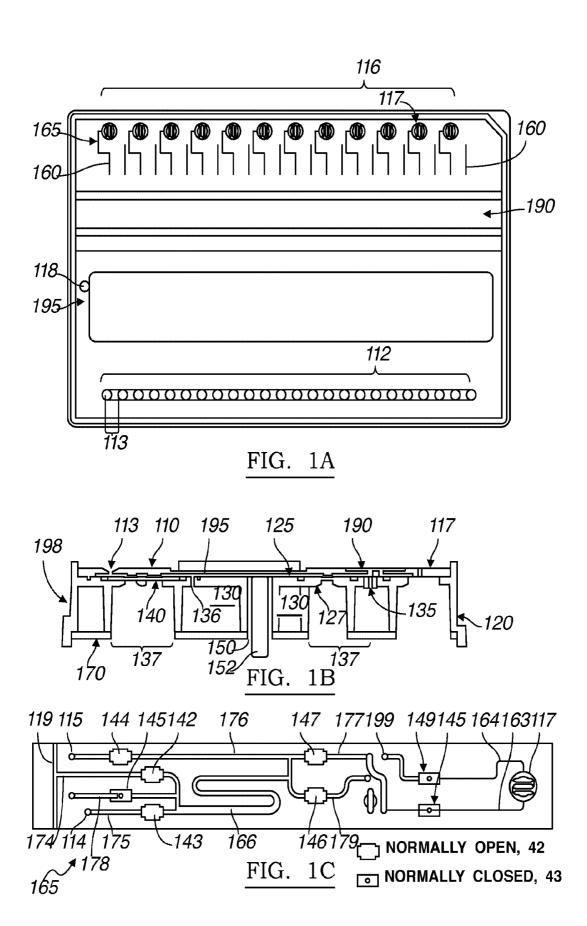


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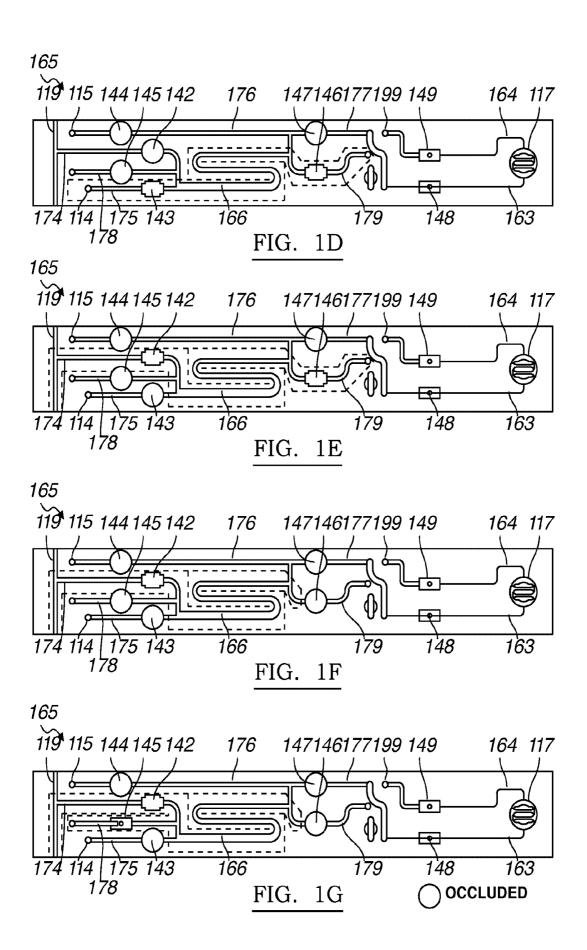
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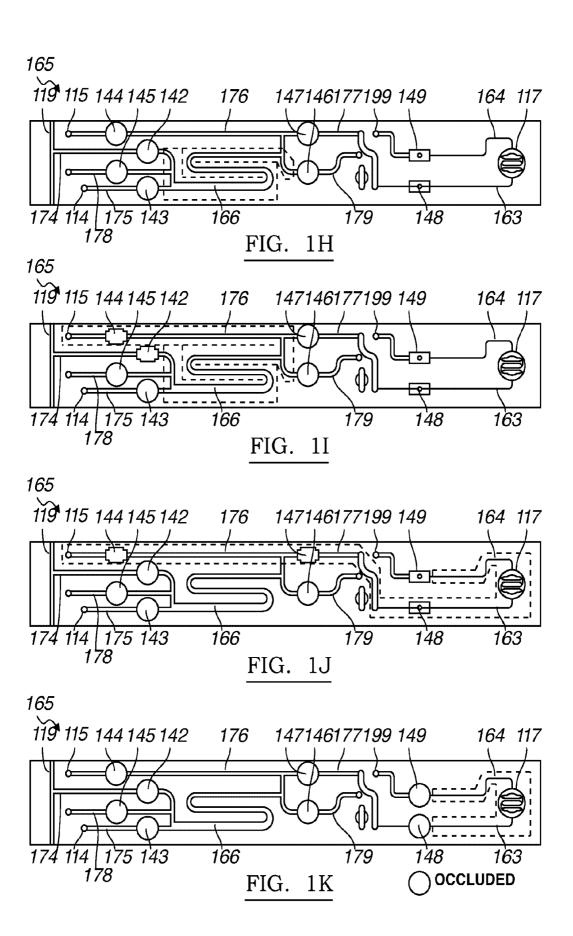
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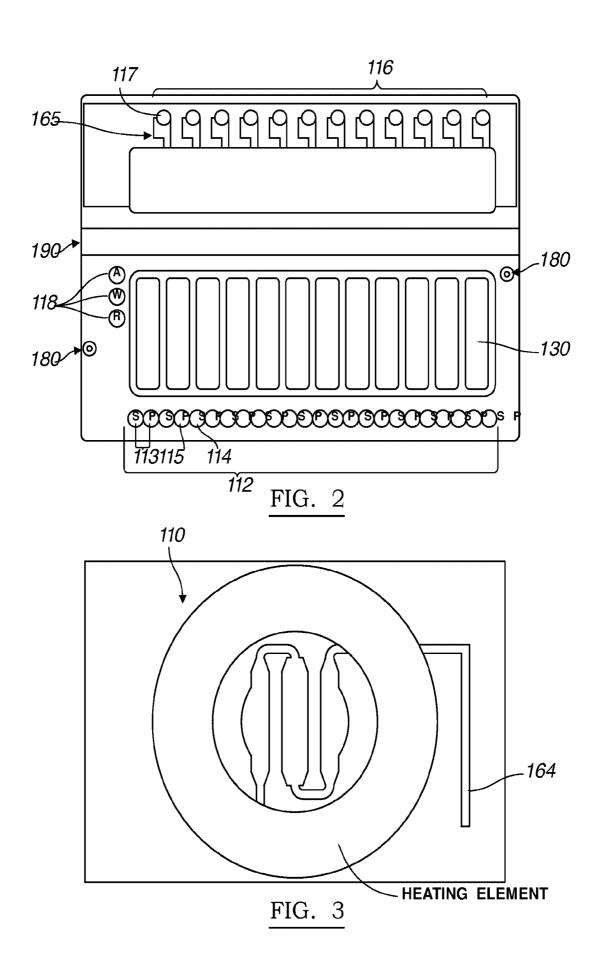
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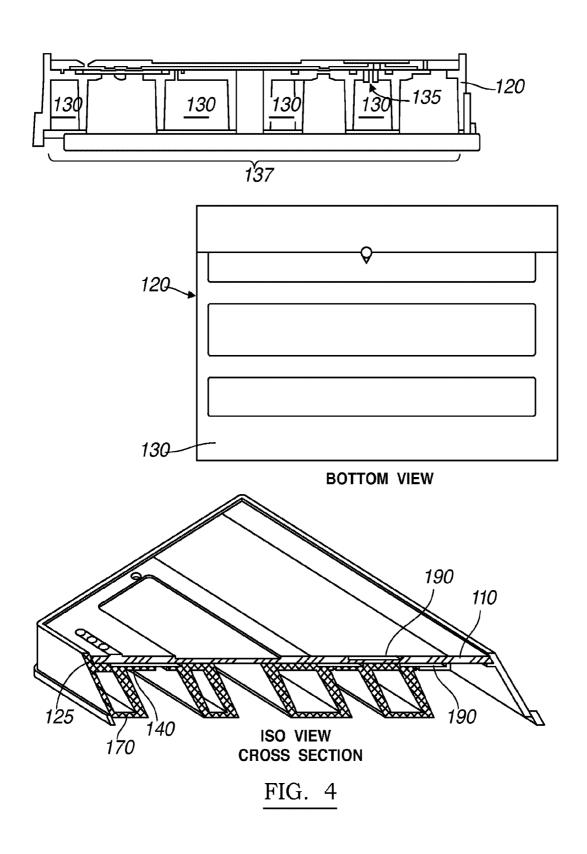
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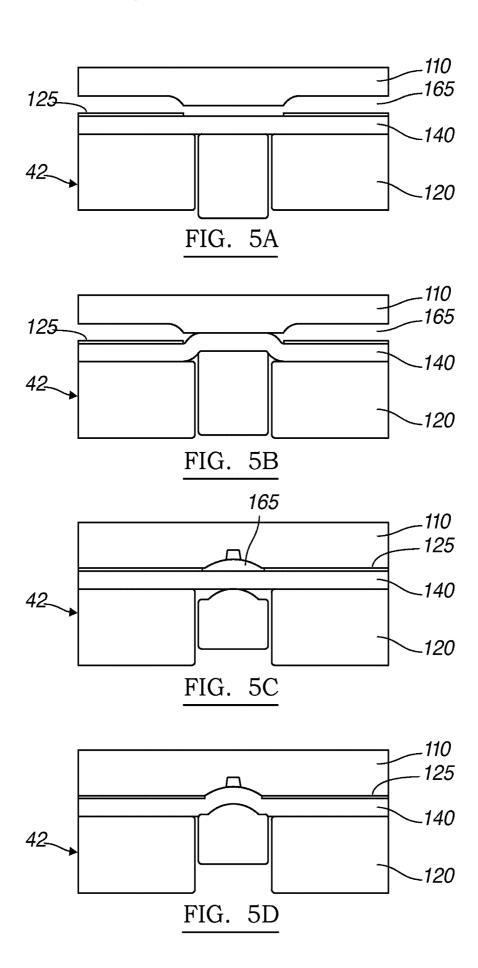
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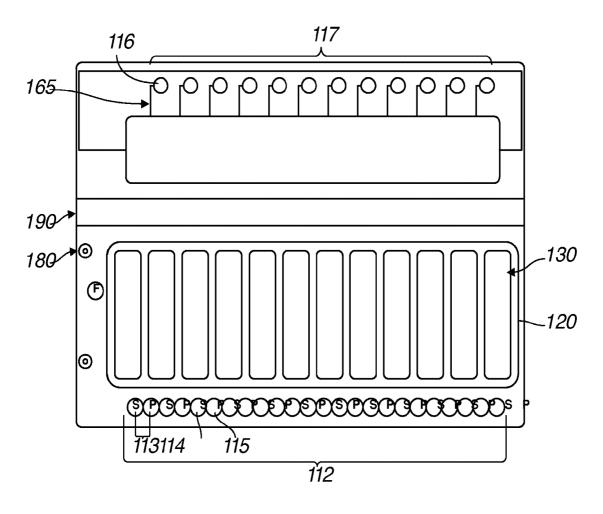
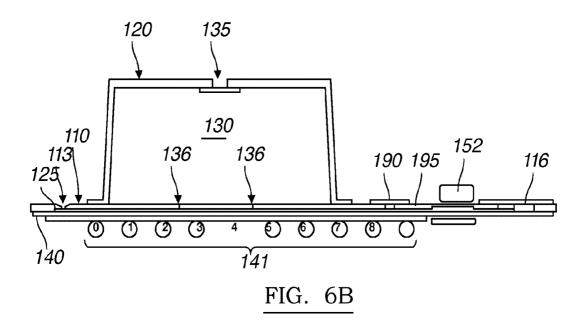


FIG. 6A

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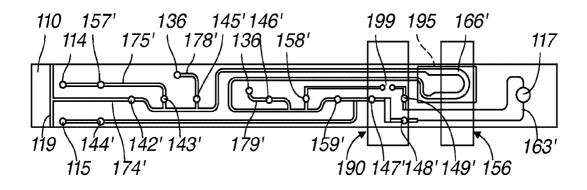


FIG. 6C

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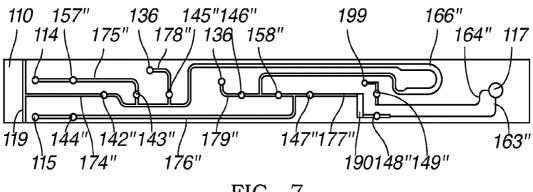
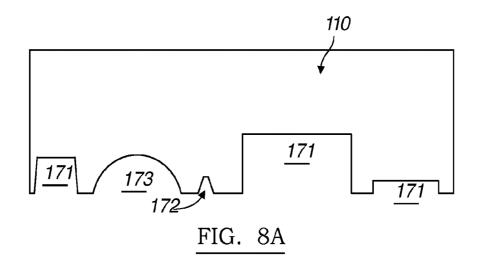


FIG. 7



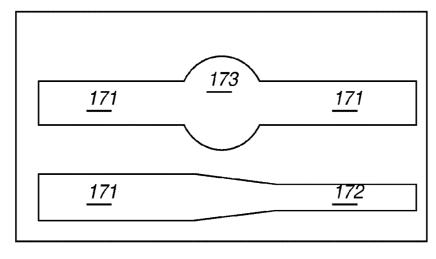
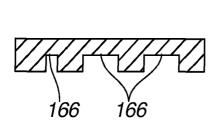


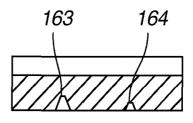
FIG. 8B

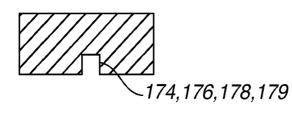
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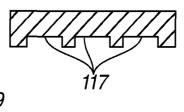
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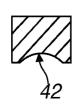
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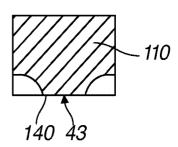












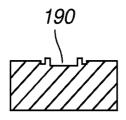
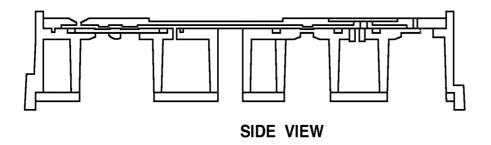


FIG. 8C

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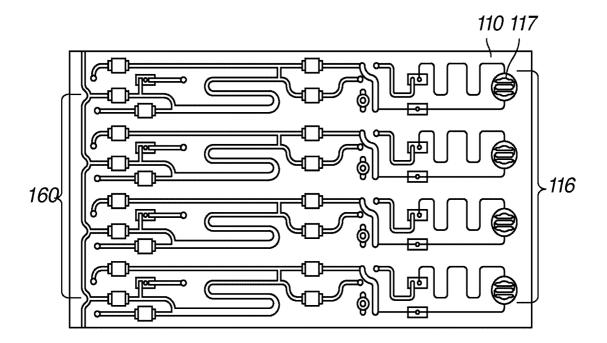


FIG. 9
TOP VIEW

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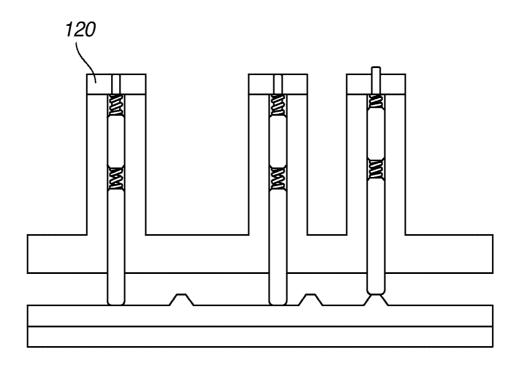
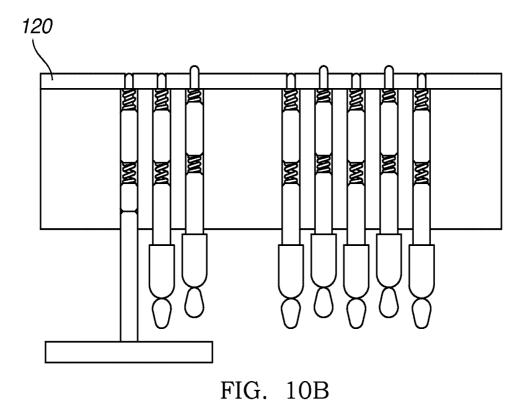
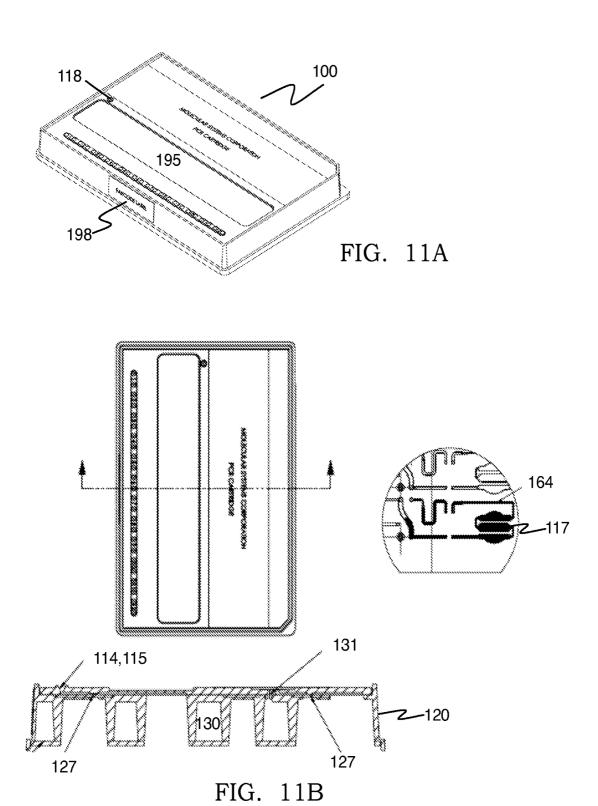


FIG. 10A



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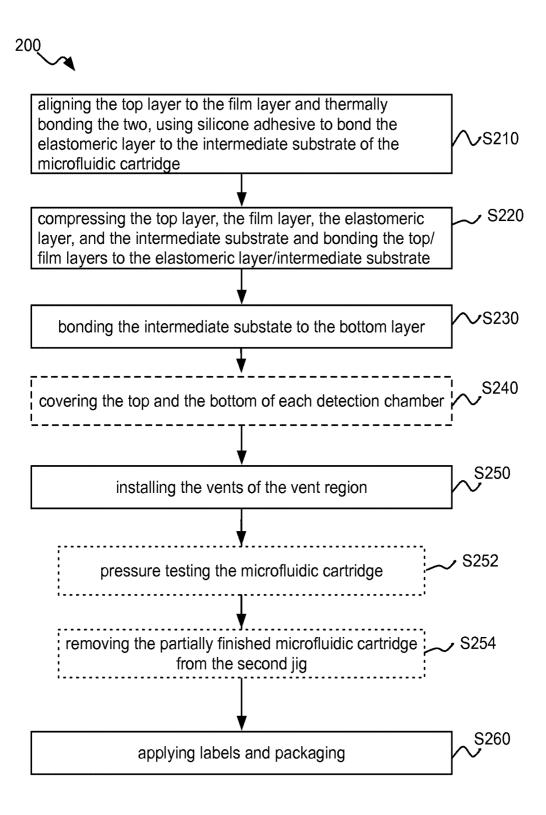
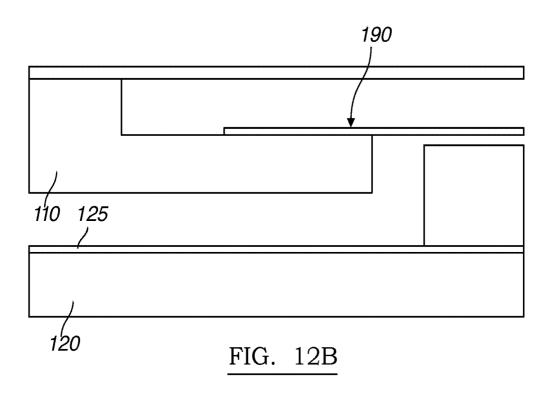


FIG. 12A

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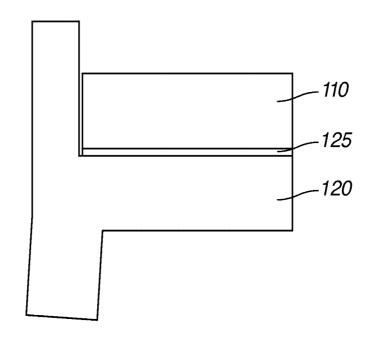
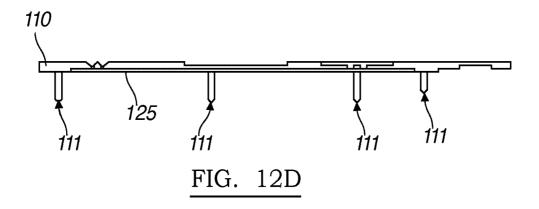
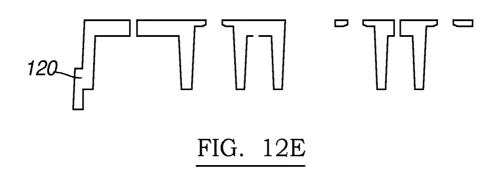


FIG. 12C

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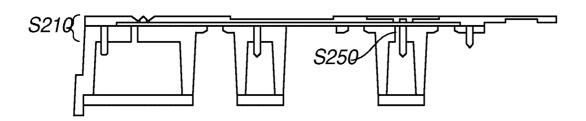


FIG. 12F

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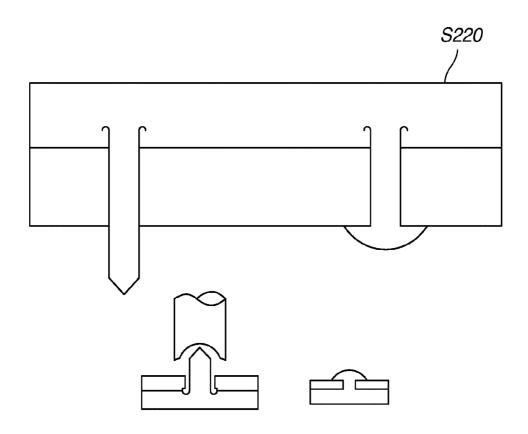


FIG. 12G

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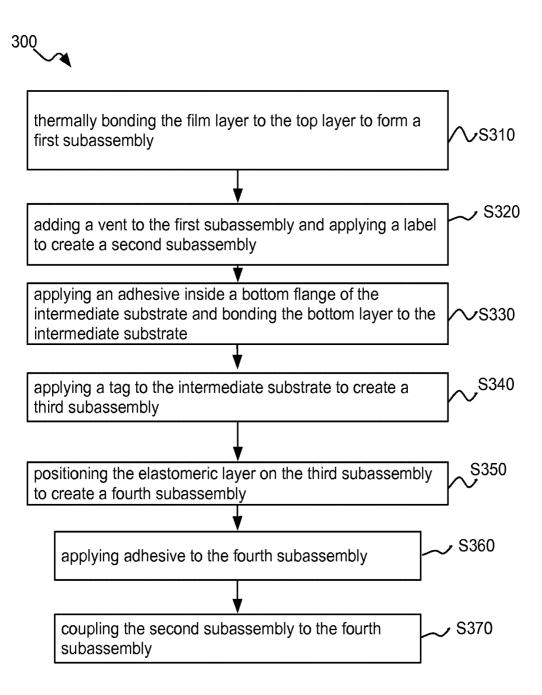


FIG. 13

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#### MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are incorporated in their entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved microfluidic cartridge for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology research proce- 25 dures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields requiring nucleic acid analysis. Molecular diagnostic analysis of biological samples 30 can include the detection and/or monitoring of one or more nucleic acid materials present in the specimen. The particular analysis performed may be either qualitative and/or quantitative. Methods of analysis may involve isolation, purification, and amplification of nucleic acid materials, and poly-35 merase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecu- 40 lar diagnostic techniques are also labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are often specific to certain nucleic acid types and not applicable across multiple acid types. Due to these 45 and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for improved devices for processing and amplifying nucleic acids. Thus, there is a need in the molecular diagnostics field to create an improved microfluidic cartridge to facilitate processing and detecting of 50 nucleic acids. This invention provides such a microfluidic cartridge.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1C depict an embodiment of a microfluidic cartridge (top and side views) and an embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. 1D-K depict an example embodiment of subsets of occlusion positions defining truncated portions of a fluidic 60 pathway;

FIG. 2 depicts an alternative embodiment of a microfluidic cartridge (top view) showing individual waste chambers located on the top of cartridge and multiple fluid ports;

FIG. 3 depicts an alternative embodiment of a detection 65 chamber of the microfluidic cartridge (top view) and a heating element configured to heat the detection chamber;

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FIG. 4 depicts an embodiment of a waste chamber of the microfluidic cartridge;

FIGS. 5A-5D depict embodiments of the elastomeric layer of the microfluidic cartridge, in open and occluded configurations:

FIGS. **6**A-**6**C depict an alternative embodiment of a microfluidic cartridge (top and side views) and an alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIG. 7 depicts another alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. **8**A and **8**B depict schematics of microfluidic channel cross sections;

FIG. **8**C depicts specific embodiments of microfluidic thannel cross sections;

FIG. 9 depicts an embodiment of the microfluidic cartridge with twelve fluidic pathways (four of which are shown);

FIGS. 10A and 10B depict embodiments of occlusion of fluidic pathways with the elastomeric layer and a valving mechanism;

FIGS. 11A and 11B depict an embodiment of the microfluidic cartridge;

FIGS. 12A-12G depict an example manufacturing method for an embodiment of the microfluidic cartridge; and

FIG. 13 depicts an alternative example manufacturing method for an embodiment of the microfluidic cartridge.

### DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

#### 1. Microfluidic Cartridge

As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample portreagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field **156**; and a set of fluidic pathways **160**, each formed by at least a portion of the top layer no, a portion of the film layer 125, and a portion of the elastomeric layer 140. In other embodiments, the microfluidic cartridge 100 may further comprise a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber 130. Furthermore, the top layer 110 of the microfluidic cartridge 100 may further comprise a shared fluid port 118, a vent region 190, and a heating region 195, such that each fluidic pathway 165 in the set of fluidic pathways 160 is fluidically coupled to a sample port-reagent port pair 113, the shared fluid port 118, the waste chamber 130, and a detection chamber 117, comprises a capture segment 166 configured to pass through the heating region and the magnetic field, and is configured to pass through the vent region 190 upstream of the detection chamber 117. Each fluidic pathway 165 thus functions to receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 165. As configured, the microfluidic cartridge 100 can be used to facilitate molecular diagnostic processes and techniques, and preferably conforms to microtiter plate dimensional standards. Alternatively, the microfluidic cartridge 100 may be any appropriate size. In a

specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample containing nucleic acids.

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1.1 Microfluidic Cartridge—Top Layer

The top layer 110 of an embodiment of the microfluidic 5 cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 10 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or 15 other amplification techniques. Preferably, the top layer 110 is composed of a polypropylene-based polymer, but the top layer no may alternatively be composed of any appropriate material (e.g. cyclic olefin polymer). In a specific embodiment, the top layer no is composed of 1.5 mm thick polypro- 20 pylene produced by injection molding, with a glass transition temperature between 136 and 163° C. The top layer no may alternatively be composed of any appropriate material, for example, a polypropylene based polymer. As shown in FIGS. 1B and 1C, the top layer no preferably comprises a set of 25 cartridge functions to receive at least one of a wash fluid, a sample port-reagent port pairs 112, a fluid port 118, a vent region 190, a heating region 195 crossing a capture segment 166 of a fluidic pathway 165, and a set of detection chambers 116.

Each sample-port-reagent port pair 113 of an embodiment 30 of the top layer no comprises a sample port 114 and a reagent port 115. The sample port 114 functions to receive a volume of a sample fluid potentially containing the nucleic acids of interest for delivery of the volume of fluid to a portion of a fluidic pathway 165 coupled to the sample port-reagent port 35 pair 113. In a specific embodiment, the volume of a sample fluid is a biological sample with magnetic beads for nucleic acid isolation; however, the volume of fluid comprising a sample fluid may alternatively be any appropriate fluid containing a sample with nucleic acids. Preferably, each sample 40 port 114 is isolated from all other sample ports, in order to prevent cross-contamination between samples of nucleic acids being analyzed. Additionally, each sample port 114 is preferably of an appropriate geometric size and shape to accommodate a standard-size pipette tip used to deliver the 45 volume of a sample fluid without leaking. Alternatively, all or a portion of the sample ports 114 are configured to be coupled to fluid conduits or tubing that deliver the volume of a sample

Each sample-port reagent port pair 113 of an embodiment 50 of the top layer 110 also comprises a reagent port 115, as shown in FIG. 1A. The reagent port 115 in a sample portreagent port pair 113 functions to receive a volume of fluid comprising a reagent used in molecular diagnostics, for delivery of the volume of fluid comprising a reagent to a portion of 55 a fluidic pathway 165 coupled to the sample port-reagent port pair 113. In a specific embodiment, the volume of fluid comprising a reagent used in molecular diagnostics is a sample of reconstituted molecular diagnostic reagents mixed with nucleic acids released and isolated using the microfluidic 60 cartridge 100; however, the volume of fluid comprising a reagent used in molecular diagnostics may alternatively be any appropriate fluid comprising reagents used in molecular diagnostics. Preferably, each reagent port 115 is isolated from all other reagent ports, in order to prevent cross-contamina- 65 tion between samples of nucleic acids being analyzed. Additionally, each reagent port 115 is preferably of an appropriate

geometric size to accommodate a standard-size pipette tip used to deliver the volume of fluid comprising a reagent used in molecular diagnostics. Alternatively, all or a portion of the reagent ports 115 are configured to be coupled to fluid conduits or tubing that deliver the volume of fluid comprising a reagent used in molecular diagnostics.

Preferably, the set of sample port-reagent port pairs 112 is located near a first edge of the top layer no, such that the configuration of the sample port-reagent port pairs 112 functions to increase accessibility, for instance, by a pipettor delivering fluids to the microfluidic cartridge 100. In one specific example, the microfluidic cartridge 100 is configured to be aligned within a module, with the set of sample portreagent port pairs 112 accessible outside of the module, such that a multichannel pipette head can easily access the set of sample port-reagent port pairs 112. Preferably, as shown in FIG. 1A, the set of sample port-reagent port pairs 112 is configured such that the sample ports 114 and the reagent ports 115 alternate along the first edge of the top layer 110. In an alternative embodiment, the set of sample port-reagent port pairs 112 may not be located near an edge of the top layer 110, and may further not be arranged in an alternating fash-

The fluid port 118 of the top layer 110 of the microfluidic release fluid, and a gas used in a molecular diagnostic procedure, such as PCR. In an embodiment, the wash fluid, the release fluid, and/or the gas are common to all samples being analyzed during a run of the diagnostic procedure using the microfluidic cartridge 100; in this embodiment, as shown in FIG. 1A, the fluid port 118 is preferably a shared fluid port, fluidically coupled to all fluidic pathways 165 coupled to the sample port-reagent port pairs 112, and configured to deliver the same wash fluid, release fluid, and/or gas through the shared fluid port. Alternatively, as shown in FIG. 2, the top layer may comprise more than one fluid port 118, configured to deliver different wash fluids, release fluids, and/or gases to individual or multiple fluidic pathways 165 coupled to the set of sample port-reagent port pairs 112.

Preferably, the fluid port 118 is located along an edge of the microfluidic cartridge 100, which functions to increase accessibility to the fluid port by a system delivering fluids to the fluid port 118. In a specific embodiment, as shown in FIG. 1A, the fluid port is located approximately midway along an edge of the microfluidic cartridge 100, different from the edge along which the set of sample port-reagent port pairs 112 is located. Alternatively, the fluid port 118 may not be located along an edge of the microfluidic cartridge 100. Additionally, the fluid port 118 is preferably configured to be coupled to a syringe pump for fluid delivery; however, the fluid port 118 may alternatively configured to couple to any appropriate system for fluid delivery. Preferably, the wash fluid is a wash buffer for washing bound nucleic acid samples (i.e. nucleic acids bound to magnetic beads), the release fluid is a reagent for releasing bound nucleic acids samples from the magnetic beads, and the gas is pressurized air for moving fluids and demarcating separate reagents. Alternatively, the wash fluid, release fluid, and gas may be any appropriate liquids or gases used to carry out a molecular diagnostic procedure.

The heating region 195 of the top layer no functions to accommodate and position a heating element relative to elements of the microfluidic cartridge 100. The heating element preferably heats a defined volume of fluid and the magnetic beads, which has traveled through the microfluidic cartridge 100, according to a specific molecular diagnostic procedure protocol (e.g. PCR protocol), and is preferably an element external to the microfluidic cartridge 100; alternatively, the

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heating element may be integrated with the microfluidic cartridge and/or comprise a thermally conductive element integrated into the microfluidic cartridge 100. The heating region 195 is preferably a recessed fixed region of the top layer 110, downstream of the sample port-reagent port pairs 112, as shown in FIGS. 1A and 1B. Alternatively, the heating region may not be fixed and/or recessed, such that the heating region 195 sweeps across the top layer 110 of the microfluidic cartridge 100 as the heating element is moved. The microfluidic cartridge 100 may altogether omit the heating region 195 of the top layer 110, in alternative embodiments using alternative processes (e.g. chemical methods) for releasing nucleic acids from nucleic acid-bound magnetic beads.

The vent region 190 of an embodiment of the top layer 110 functions to remove unwanted gases trapped within a fluidic 15 pathway 165 of the microfluidic cartridge, and may additionally function to position a defined volume of fluid within a fluidic pathway 165 of the microfluidic cartridge. The vent region 190 is preferably located downstream of the heating region 195 in an embodiment where the heating region 195 is 20 fixed on the top layer 110 of the microfluidic cartridge 100, but alternatively may be located at another appropriate position on the top layer 110 such that unwanted gases are substantially removed from the microfluidic cartridge 100 during analysis. The top layer no may alternatively comprise more 25 than one vent region 190 located at appropriate positions in the top layer 110. Preferably, as shown in FIGS. 1A and 1B, the vent region 190 is a recessed region in the top layer no, and further comprises a film covering the vent region 190. Preferably, the film covering the vent region 190 is a gas-permeable but liquid-impermeable film, such that unwanted gases may be released from the microfluidic cartridge 100, but fluids remain within the microfluidic cartridge 100 and flow to the point of contacting the film. This functions to remove unwanted gases and position a defined volume of fluid within 35 a fluidic pathway 165 of the microfluidic cartridge. In a specific embodiment, the film covering the vent region is a hydrophobic porous polytetrafluoroethylene-based material, synthesized to be gas-permeable but liquid-impermeable. Alternatively, the film covering the vent region may be gas 40 and liquid permeable, such that unwanted gases and liquids are expelled from the microfluidic cartridge 100 through the vent region 190. Other alternative embodiments of the microfluidic cartridge 100 may altogether omit the vent region.

The set of detection chambers 116 of an embodiment of the top layer no functions to receive a processed nucleic acid sample, mixed with molecular diagnostic reagents, for molecular diagnostic analysis. Preferably, the set of detection chambers 116 is located along an edge of the top layer no, 50 opposite the edge along which the set of sample port-reagent port pairs 112 is located, which allows sample fluids dispensed into the microfluidic cartridge 100 to be processed and mixed with molecular diagnostic reagents on their way to a detection chamber 117 of the set of detection chambers 116 55 and facilitates access to the detection chambers by external elements performing portions of a molecular diagnostics protocol (e.g. heating and optics systems). Alternatively, the set of detection chambers 116 may not be located along an edge of the top layer 110. In a first variation, as shown in FIGS. 1A 60 and 11B, each detection chamber 117 in the set of detection chambers comprises a serpentine-shaped channel 16 for facilitating analysis of a solution of nucleic acids mixed with reagents. In the first variation, three portions of the serpentine-shaped channel 16 are preferably wide and shallow to 65 facilitate heating, and are interconnected by two narrow portions, which function to increase fluid flow resistance and

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reduce the proportion of nucleic acid not contained within the detection area. The first variation functions to facilitate filling of the set of detection chambers in a manner that reduces the potential for trapped air bubbles, to facilitate rapid molecular diagnostic techniques, and to comply with current imaging technologies. In a specific example of the first variation, each serpentine-shaped channel 16 is injected molded into the top layer 110 of the microfluidic cartridge 100, and the three interconnected portions of the serpentine-shaped channel 16 are each 1600  $\mu$ m wide by 400  $\mu$ m deep.

In a second variation, each detection chamber 117 in the set of detection chambers has a depth between 0.400 mm and 1.00 mm, and a diameter between 3.50 mm and 5.70 mm, to provide a volumetric configuration that facilitates reaction efficiency. In a specific example of the second variation, each detection chamber 117 in the set of detection chambers 116 is configured to contain a total volume of 10 uL, and has a depth of 0.80 mm and a diameter of 3.99 mm; however, in alternative embodiments, each detection chamber 117 in the set of detection chambers 116 may be configured to contain a total volume less than or greater than 10 uL.

Preferably, as shown in FIGS. 1A and 1B, the lower regions of each detection chamber 117 in the set of detection chambers 116 includes a PCR compatible film that is thin, to facilitate efficient thermocycling, and has low autofluorescence, to facilitate light-based molecular diagnostic assays performed at the set of detection chambers 116. The PCR compatible film is preferably composed of a polypropylene based polymer thermally bonded to the bottom of the top layer, but may alternatively be composed of any appropriate PCR-compatible material and bonded in any fashion. In one specific variation, the PCR compatible film is a cyclic olefin polymer (COP) film, thermally bonded to the top layer 110, with a glass transition temperature suitable for a molecular diagnostic protocol. In one alternative embodiment, depending on the configuration of imaging, heating, and/or cooling elements external to the microfluidic cartridge 100, the top and/or bottom of the detection chambers 117 in the set of detection chambers 116 may be entirely formed of a clear or transparent material (e.g. glass or plastic) allowing transmission of light. In a variation of this alternative embodiment, lensing, other optical components, or additional structures may also be incorporated into the detection chambers, to facilitate light transmission and/or focusing. In the variation of the alternative embodiment, a lens may be manufactured (e.g. injection molded) directly to form a surface of a detection chamber 117.

In the embodiment of the set of detection chambers 116 that includes a PCR compatible film, the PCR compatible film may further include a thermally conductive component, which functions to transfer heat from a heating element to the detection chamber. Depending on the position of the heating element(s) relative to the microfluidic cartridge 100 during analysis, the thermally conductive component of the PCR compatible film may be integrated with just the upper region of each detection chamber, just the lower region of each detection chamber, or both the upper and lower regions of each Detection chamber. The thermally conductive component of the PCR compatible film may comprise a wire mesh with a substantially small wire diameter, as shown in FIG. 3, thermally conductive particles distributed through the PCR compatible film (in a manner that still allows for optical clarity), or any other appropriate thermally conductive component (e.g. thermally conductive beads integrated into the PCR compatible film). The region laterally around the detection chamber may also further include one or more heattransfer elements or air channels speed heat dissipation.

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Alternatively, a detection chamber 117 in the set of detection chambers 116 may not include a PCR compatible film with a thermally conductive component. Preferably, each detection chamber 117 is heated using a diced silicon wafer with conductive channels flip-chip bonded to a detection chamber to provide resistive heating; however, each detection chamber 117 may alternatively be heated using any appropriate heating device or method, and may be assembled using any appropriate method.

Preferably, each detection chamber 117 in the set of detection chambers 116 is thermally isolated from all other detection chambers, in order to prevent contamination of data from a detection chamber 117 due to heat transfer from other detection chambers in the set of detection chambers 116. In one embodiment, each detection chamber 117 of the set of 15 detection chambers 116 is spaced far from adjacent detection chambers to limit thermal crosstalk. In another alternative embodiment, the top layer 110 may comprises slots between adjacent detection chambers to separate the detection chambers with an air gap. In one variation, thermal isolation is 20 achieved by surrounding the side walls of each detection chamber 117 with a thermally insulating material, such as an insulating epoxy, putty, filler, or sealant. In another variation, the thermally insulating material has a low density, which functions to reduce heat transfer from other detection cham- 25 bers. In yet another variation, thermal isolation is achieved by geometrically separating or displacing the detection chambers relative to each other within the top layer 110 of the microfluidic cartridge 100, such that heat transfer between detection chambers is hindered.

Preferably, each detection chamber 117 in the set of detection chambers 116 is also optically isolated from all other detection chambers, in order to prevent contamination of data from a detection chamber 117 due to light transfer from other detection chambers in the set of detection chambers 116. 35 Preferably, optical isolation is achieved with detection chambers having substantially vertical walls, and separating each detection chamber 117 in the set of detection chambers from each other. However, in one variation, the sidewalls of each detection chamber 117 in the set of detection chambers 116 40 are either composed of or surrounded by a material with low autofluorescence and/or poor optical transmission properties to achieve optical isolation. In another variation, the sidewalls of each detection chamber 117 are surrounded by an optically opaque material, thus allowing transmission of light to a 45 detection chamber 117 through only the top and bottom regions of the detection chamber 117. Alternatively, the microfluidic cartridge 100 may not further comprise any provisions for optical isolation of each detection chamber 117 in the set of detection chambers 116, aside from constructing the 50 set of detection chambers 116 with a material having low autofluorescence.

Additionally, each detection chamber 117 in the set of detection chambers 116 may be further optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical detection, and facilitate filling in a manner that limits bubble generation. Alternatively each detection chamber 117 in the set of detection chambers 116 may not be optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical 60 detection, and/or facilitate filling in a manner that limits bubble generation.

The top layer 110 of the microfluidic cartridge 100 may further comprise a set of cartridge-aligning indentations 180, which function to align the microfluidic cartridge 100 as it 65 moves through an external module. As shown in FIG. 2 the set of cartridge-aligning indentations 180 are preferably located

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such that they do not interfere with any ports 112, 118, the heating region, 195, the vent region 190, and/or the set of detection chambers 116. In an embodiment, the top layer 110 of the microfluidic cartridge preferably comprises at least four cartridge-aligning indentations, located at points on the periphery of the top layer 110, and the cartridge-aligning indentations are configured to be recessed regions configured to mate with alignment pins in a system external to the microfluidic cartridge 100. Alternatively, the cartridge-aligning indentations may be grooves, such that the microfluidic cartridge 100 accurately slides into position along the grooves within a system external to the microfluidic cartridge 100. In yet another alternative embodiment, the set of cartridgealigning indentations 180 may be any appropriate indentations that allow for positioning of the microfluidic cartridge 100 within an external system. However, the microfluidic cartridge 100 may altogether omit the set of cartridge-aligning indentations 180, and rely upon other features of the microfluidic cartridge 100 to facilitate alignment.

1.2 Microfluidic Cartridge—Intermediate Substrate

As shown in FIG. 1B, an embodiment of the microfluidic cartridge also comprises an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer no by a film layer 125, configured to form a waste chamber 130. The intermediate substrate 120 functions to serve as a substrate to which layers of the microfluidic cartridge may be bonded, to provide guides for the valve pins, and to provide a waste chamber volume into which a waste fluid may be deposited. Preferably, the depth of the intermediate substrate 120 provides a waste chamber volume adequate to accommodate the volume of waste fluids generated within the microfluidic cartridge 100. Additionally, the depth of the intermediate substrate 120 provides a low profile for the microfluidic cartridge 100 to facilitate movement throughout a compact molecular diagnostic system. Preferably, the intermediate substrate 120 of the microfluidic cartridge 100 is also configured such that the footprint of microfluidic cartridge 100 adheres to microtiter plate standards, to facilitate automated handling of the microfluidic cartridge 100. The intermediate substrate 120 is preferably composed of a low-cost, structurally stiff material, such as polypropylene. However, similar to the top layer 120, the intermediate substrate may be alternatively composed of a structurally stiff material with low autofluorescence, such that the intermediate substrate 120 does not interfere with sample detection by fluorescence techniques, and an appropriate glass transition temperature for PCR techniques. In one variation of this alternative embodiment, the intermediate substrate 120 is composed of a cyclic olefin polymer (COP), produced by injection molding, with a glass transition temperature between 136 and 163° C. In yet another alternative embodiment, the intermediate substrate 120 may be composed of any appropriate material, for example, a polycarbonate based polymer.

Preferably, the intermediate substrate 120 of the microfluidic cartridge 100 is coupled to the top layer no and partially separated from the top layer 110 by a film layer 125. The film layer 125 functions to isolate individual fluidic pathways 165 of the microfluidic cartridge, to prevent leakage, to provide an appropriate environment for sample processing and conducting a molecular diagnostic protocol, and to provide access between a microfluidic channel (of a fluidic pathway 165) above the film layer 125 and elements below the film layer 125 (e.g. waste chamber and/or fluidic pathway occluder). Preferably, the film layer is a polypropylene (PP) with an appropriate glass transition temperature, such that it is PCR compatible and thermally bondable to the top layer 110;

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however, the film layer may alternatively be any appropriate material. In a specific embodiment, the film layer 125 is a polypropylene film between 30 and 100 microns thick and die cut to produce openings at a set of occlusion positions, to provide access between a microfluidic channel of a fluidic 5 pathway 165 above the film layer 125 and elements below the film layer 125. In this specific embodiment, the openings are slightly oversized prior to assembly, in order to allow for constriction during assembly (due to thermal and pressure effects) and to provide higher tolerance during assembly of 10 microfluidic cartridge layers. Alternatively, the film layer is any appropriate material such that it substantially isolates individual fluidic pathways, and is easily processable to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer and elements below the film 15 layer **125** 

Preferably, the top layer 110, the film layer 125, and the intermediate substrate are bonded together, such that the top layer 110, film layer, 125, and intermediate substrate form a bonded unit with a hermetic seal to prevent fluid leakage. A 20 hermetic seal is preferably formed using a silicone rubber layer coupled to the film layer 125, but may alternatively be formed using an alternative material or method. In a specific embodiment, a hermetic seal formed using a silicone rubber layer is only required at locations of openings within the film 25 layer (e.g, at locations where an external occluder interacts with the microfluidic cartridge). Preferably, in an embodiment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially identical materials (e.g. polypropylene), at least one of thermal bonding, 30 adhesives, and ultrasonic welding are used to coupled the layers 110, 125, 120 together. In an embodiment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially different materials—a combination of thermal bonding methods and adhesives may be used 35 to bond the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 together. In an alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 may be thermally bonded together 40 in a single step. In yet another alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 may alternatively be modular, in applications where a portion of the microfluidic cartridge 100 is partially reusable (e.g. in an application where the waste chamber may be 45 discarded after use, but the top layer and film may be reused). In yet another alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 may only be partially bonded, such that a molecular diagnostic system, into which the microfluidic cartridge 100 is loaded, is con- 50 figured to compress the top layer 110, the film layer 125, and the intermediate substrate 120 together, preventing any fluid

As shown in FIG. 1B, the intermediate substrate 120 of an embodiment of the microfluidic cartridge 100 is configured to 55 form a waste chamber 130, which functions to receive and isolate waste fluids generated within the microfluidic cartridge 100. The waste chamber 130 is preferably continuous and accessible by each fluidic pathway 165 of the microfluidic cartridge 100, such that all waste fluids generated within 60 the microfluidic cartridge 100 are deposited into a common waste chamber; however, each fluidic pathway 165 of the microfluidic cartridge 100 may alternatively have its own corresponding waste chamber 130, such that waste fluids generated within a fluidic pathway 165 of the microfluidic cartridge 100 are isolated from waste fluids generated within other fluidic pathways 165 of the microfluidic cartridge 100.

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In a specific embodiment of the microfluidic cartridge 100 with a continuous waste chamber, the waste chamber has a volumetric capacity of approximately 25 mL; however, the waste chamber 130 of another embodiment may have a different volumetric capacity. The intermediate substrate 120 further comprises a waste vent 135, which provides access between a microfluidic channel of a fluidic pathway 165 above the film layer 125 and the waste chamber 130. Preferably, the intermediate substrate 130 comprises more than one waste inlet 136, such that the waste chamber is accessible at more than one location along a fluidic pathway 165 through the waste inlets 136. Alternatively, the intermediate substrate 120 may include a single waste inlet 136, such that all waste fluids generated within the microfluidic cartridge 100 are configured to travel through the single waste inlet 136 into the waste chamber 130. Also, as shown in FIG. 1B, the intermediate substrate 120 may comprise a waste vent 131, such that the waste chamber 130 is vented to prevent pressure build up in the waste chamber as waste fluid is added.

As shown in FIGS. 1B and 4, the waste chamber 130 formed by the intermediate substrate 120 preferably has a corrugated surface 137, such that the waste chamber 130 is not only configured to receive and isolate a waste fluid, but also functions to 1) provide structural stability for the microfluidic cartridge 100 and 2) allow elements external to the microfluidic cartridge 100 to enter spaces formed by the corrugated surface 137, for greater accessibility to elements of the microfluidic cartridge 100. Also shown in FIGS. 1B and 4, each of the ridges in the corrugated surface 137 may not have the same dimensions, as a result of the locations of elements within and external to the microfluidic cartridge 100. In an embodiment of the waste chamber 130 with a corrugated surface 137, at least two ridges of the corrugated surface 137 are preferably the same height, such that the microfluidic cartridge 100 sits substantially level on a flat base. In an alternative embodiment, all ridges of the corrugated surface 137 of the waste chamber 130 are identical, for structural symmetry, and in yet another embodiment, the waste chamber 130 may not have a corrugated surface 137.

In one preferred embodiment, the intermediate substrate 120 of the microfluidic cartridge 100 further comprises a set of valve guides, which function to direct a series of external pins or other indenters through the valve guides at a set of occlusion positions 141, thus affecting flow through a microfluidic channel of a fluidic pathway 165 at the set of occlusion positions 141. The set of valve guides 127 may also function to facilitate alignment of the microfluidic cartridge 100 within an external molecular diagnostic module. In a first embodiment, as shown in FIG. 1B, the set of valve guides 127 comprises holes within the intermediate substrate 120 at the set of occlusion positions 141, with sloped edges configured to direct a pin or indenter through the holes. In the first embodiment, the set of valve guides 127 may be produced in the intermediate substrate 120 by injection molding, or may alternatively be produced by drilling, countersinking, chamfering, and/or beveling. In another embodiment, the set of valve guides 127 comprises grooves with holes, such that a pin or indenter is configured to travel along a groove and through a hole that defines the valve guide. In a simplified alternative variation, the set of valve guides 127 may comprise holes through the intermediate substrate 120, wherein the holes do not have sloped edges. In yet another simplified alternative variation, the set of valve guides 127 may comprise a slot configured to provide access to the elastomeric layer 140 by a group of occluding objects (e.g. pins or indenters), rather than a single occluding object.

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1.3 Microfluidic Cartridge—Elastomeric and Bottom Layers As shown in FIGS. 1B and 5A-5D, an embodiment of the microfluidic cartridge 100 also comprises an elastomeric layer 140 partially situated on the intermediate substrate 120, which functions to provide a deformable substrate that, upon 5 deformation, occludes a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 at an occlusion position of a set of occlusion positions 141. Preferably, the elastomeric layer 140 comprises an inert, liquid impermeable material, of an appropriate thickness, that can be 10 heated to temperatures encountered during manufacturing and/or specified in a molecular diagnostic protocol, without substantial damage (i.e. compromised surface and/or loss of mechanical robustness) and is chemically compatible with a PCR assay. Preferably, the elastomeric layer 140 is non- 15 continuous, such that portions of the elastomeric layer 140 are positioned relative to the intermediate substrate 120 in a manner that directly covers holes provided by the set of valve guides 127. Alternatively, the elastomeric layer 140 is a continuous layer, spanning a majority of the footprint of the 20 microfluidic cartridge 100 while covering holes provided by the set of valve guides 127. In a specific embodiment, the elastomeric layer 140 comprises 500 micron thick strips of a low-durometer silicone that can be heated to at least 120° C. without substantial damage, which are bonded to a portion of 25 the intermediate substrate 120 using a silicone-based adhesive and slightly compressed between the film layer 125 and the intermediate substrate 120. In a variation of the specific embodiment, the elastomeric layer 140 may alternatively be held in place solely by pressure between the intermediate 30 layer 120 and the top layer 110. Preferably, the elastomeric layer 140 is reversibly deformable over the usage lifetime of the microfluidic cartridge 100, such that any occlusion of a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 is reversible over the usage lifetime of 35 the microfluidic cartridge. Alternatively, the elastomeric layer 140 may not be reversibly deformable, such that an occlusion of a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 is not reversible.

The set of occlusion positions 141 preferably comprises at 40 least two types of occlusion positions, as shown in FIG. 1C, including a normally open position 42 and a normally closed position 43. As shown in FIGS. 5A-5D, the elastomeric layer 140 at a normally open position 42 of the set of occlusion positions 141 may be closed upon occlusion by an occluding 45 object (FIGS. 5B and 5D). Preferably, a normally open position 42 is configured to withstand pressures that can be generated by a fluid delivery system (e.g. a syringe pump) without leaking, upon occlusion by an occluding object at the normally open position 42. In one specific example, a ½ 50 barrel-shaped pin head may be used to fully occlude a normally open position 42 having an arched cross section, as in FIG. 5C, with near constant pressure on the portion of the elastomeric layer compressed between the occluding object and occluding position.

The normally closed position 43 of the set of occlusion positions 141, functions to be normally closed, but to be forced open in response to fluid delivery by a fluid delivery system. In one variation, the normally closed position 43 may be formed by manufacturing (e.g. injection molding) the top 60 layer 100, such that the top layer material at a normally closed position 43 extends down to the elastomeric layer 140. If an occluding object is held away from the normally closed position 43, the occlusion position is closed, but can be forced open due to fluid pressure applied by a fluid delivery system 65 (e.g. syringe pump). When not in operation, however, the normally closed position 43 is configured to prevent leakage

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and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.

The microfluidic cartridge 100 may further comprise a bottom layer 170 configured to couple to the intermediate substrate, which functions to allow waste to be contained within the microfluidic cartridge 100, and allow microfluidic cartridges to be stacked. The bottom layer thus facilitates reception, isolation, and containment of a waste fluid within the waste chamber. Preferably, the bottom layer 170 is composed of the same material as the intermediate substrate 120 for cost and manufacturing considerations, and bonded to the intermediate substrate 120 in a manner that provides a hermetic seal, such that a liquid within the waste chamber 130 does not leak out of the waste chamber 130. In a specific embodiment, the bottom layer 170 and the intermediate substrate 120 are both composed of a polypropylene-based material, and bonded together using an adhesive. In an embodiment of the microfluidic cartridge 100 where the waste chamber 130 has a corrugated surface, the bottom layer 170 preferably only seals voids defining the waste chamber 130, such that non-waste chamber regions (i.e. non-waste housing regions) are not covered by the bottom layer 170. Alternatively, the microfluidic cartridge 100 may omit the bottom layer 170, such that any waste fluid that enters the waste chamber 130 completely leaves the microfluidic cartridge 100 and is collected off-cartridge by a waste-collecting subsystem of an external molecular diagnostic system. In this alternative embodiment, the intermediate substrate 120 is configured to fluidically couple to the waste-collecting subsystem.

#### 1.4 Microfluidic Cartridge—Magnet Housing

The magnet housing region 150 of the microfluidic cartridge 100 functions to provide access to and/or house at least one magnet 152 providing a magnetic field 156 for purification and isolation of nucleic acids. Preferably, the magnet housing region 150 is defined by the film layer and the intermediate substrate, such that the film layer and the intermediate substrate form the boundaries of the magnet housing region 150. In an embodiment of the microfluidic cartridge 100 comprising a bottom layer 170, the magnet housing region 150 may further be defined by the bottom layer 170, such that the bottom layer partially forms a boundary of the magnet housing region 150. The magnet housing region 150 is preferably a rectangular prism-shaped void in the microfluidic cartridge 150, and accessible only through one side of the microfluidic cartridge 100, as shown in FIG. 1B. Preferably, the magnet housing region 150 can be reversibly passed over a magnet 152 to house the magnet 152, and retracted to remove the magnet 152 from the magnet housing region 150; however, the magnet 152 may alternatively be irreversibly 55 fixed within the magnet housing region 150 once the magnet 152 enters the magnet housing region 150.

Preferably, the magnet housing region 150 is bounded on at least two sides by the waste chamber 130, and positioned near the middle of the microfluidic cartridge 100, such that a fluidic pathway 165 passing through the magnetic field 156 passes through the magnetic field 156 at least at one point along an intermediate portion of the fluidic pathway 165. Preferably, the magnet housing region 150 also substantially spans at least one dimension of the microfluidic cartridge, such that multiple fluidic pathways 165 of the microfluidic cartridge 100 cross the same magnet housing region 150, magnet 152, and/or magnetic field 156. Alternatively, the

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magnet housing region 150 may be configured such that a magnet within the magnet housing region 150 provides a magnetic field spanning all fluidic pathways 165 of the microfluidic cartridge in their entirety. In alternative embodiments, the microfluidic cartridge may comprise more than 5 one magnet housing region 150, a magnet housing region 150 may be configured to receive and/or house more than one magnet 152, and/or may not be positioned near the middle of the microfluidic cartridge 100. In yet another alternative embodiment, the magnet housing region 150 may permanently house a magnet 152, such that microfluidic cartridge comprises a magnet 152, integrated with the intermediate substrate 120. In embodiments where the magnet 152 is retractable from the microfluidic cartridge 100, the magnet 152 may be a permanent magnet or an electromagnet. In 15 embodiments where the magnet 152 is configured to be integrated with the microfluidic cartridge 100, the magnet 152 is preferably a permanent magnet, which provides a stronger magnetic field per unit volume.

#### 1.5 Microfluidic Cartridge—Fluidic Pathways

The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which pro- 25 cessed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/ or detection. Preferably, each fluidic pathway 165 in the set of fluidic pathways 160 is formed by at least a portion of the top layer, a portion of the film layer, and a portion of the elasto- 30 meric layer 140, such that each fluidic pathway 165 may be occluded upon deformation of the elastomeric layer 140 at a set of occlusion positions 141. Additionally, at least one fluidic pathway 165 in the set of fluidic pathways 160 is preferably fluidically coupled to a sample port-reagent port pair 113 35 of the set of sample port-reagent port pairs 112, a fluid port 118, a waste chamber 130, and a detection chamber 117 of the set of detection chambers 116. Furthermore, at least one fluidic pathway 165 in the set of fluidic pathways 160 is preferably configured to be occluded upon deformation of the 40 elastomeric layer 140, configured to transfer a waste fluid to the waste chamber 30, comprises a capture segment 166 passing through the heating region 195 and a magnetic field 156, and is configured to pass through the vent region 190 upstream of a detection chamber 117. Alternative embodi- 45 ments may omit preferred elements of the embodiment of the fluidic pathway 165 described above, such as a vent region 190 or a heating region 195, or add additional elements to the embodiment of the fluidic pathway 165 described above.

A fluidic pathway 165 of the set of fluidic pathways 160 50 may comprise portions (i.e. microfluidic channels) that are located on both sides of the top layer no, but is preferably located primarily on the bottom side of the top layer (in the orientation shown in FIG. 1B). In the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a microfluidic 55 channel on top of the top layer no may be further covered by second film layer 168 that seals the microfluidic channel on top of the top layer no. The second film layer 168 may be comprise a cyclic olefin polymer (COP) film, thermally or adhesively bonded to the top layer no, or alternatively may 60 comprise another material that is bonded to the top layer no. The use of film layers 125, 168 to cover microfluidic channels on either side of the top layer no facilitates manufacturing, such that long stretches of a fluidic pathway 165 do not need to be produced within the interior of the top layer no. Prefer- 65 ably, microfluidic channels may be etched, formed, molded, cut, or otherwise shaped into the rigid structure of the top

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layer no, and either remain on one side of the top layer no, or pass through the thickness of the top layer 110.

In one variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 11B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer no, comprising a segment running to a vent region 190 on the top side of the top layer no. All other segments of the fluidic pathway 165 are preferably located on the bottom side of the top layer no, allowing the fluidic pathway 165 to be sealed by the film layer 125 without requiring a separate film layer to seal channels located on the top of the top layer 110.

In another variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a detection chamber 163 on the top side of the top layer 110 and a segment running away from the detection chamber 164 on the top side of the top layer 110. In this variation, the fluidic pathway 165 thus crosses the thickness of the top layer no upstream of the 20 first segment running to the detection chamber 163, and crosses the thickness of the top layer 110 downstream of the segment running away from the detection chamber 164, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. In another variation, as shown in FIG. 6C, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising only a segment running away from the detection chamber 164 on the top side of the top layer 110. In this other variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 downstream of the second portion, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. Alternatively, other embodiments may comprise a fluidic pathway 165 with a different configuration of portions on the top side of the top layer no and/or portions on the bottom side of the top layer 110.

As shown in FIGS. 1C, 6C, 7 and 9, a fluidic pathway 165 of the set of fluidic pathways 160 is branched and preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118, a sample segment 175 coupled to a sample port 114, a reagent segment 176 coupled to a reagent port 115, a capture segment 166 passing through at least one of the heating region 195 and a magnetic field 156, a vent segment 177 configured to pass through the vent region 190, a segment running to a detection chamber 163, a segment running away from the detection chamber 164, and at least one waste segment 178, 179 configured to transfer a waste fluid to a waste chamber 130. Individual segments of the fluidic pathway 165 are preferably configured to pass through at least one occlusion position of the set of occlusion positions 141, to controllably direct fluid flow through portions of the fluidic pathway 165. A fluidic pathway 165 may also further comprise an end vent 199, which functions to prevent any fluid from escaping the microfluidic channel.

The initial segment 174 of the fluidic pathway 165 functions to deliver common liquids and/or gases from a fluid port 118 through at least a portion of the fluidic pathway 165, the sample segment 175 functions to deliver a volume of a sample fluid (e.g. sample comprising nucleic acids bound to magnetic beads) to a portion of the fluidic pathway 165, and the reagent segment 176 functions to deliver a volume of fluid comprising a reagent to a portion of the fluidic pathway 165. The capture segment 166 functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid, and may be s-shaped and/or progressively narrowing, to increase the efficiency and/or effectiveness of isolation and purification. Alternatively, the capture segment 166 may alto-

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gether be replaced by a substantially straight portion 166 or any other geometric shape or configuration that functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid. The capture segment 166 of the fluidic pathway 165 preferably has an aspect ratio less than 5 one, which functions to facilitate capture of magnetic particles, but may alternatively have an aspect ratio that is not less than one.

The vent segment 177 functions to deliver a processed sample fluid through the vent region 190 for gas removal. The 10 segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117. The 15 segments may be arranged in at least one of several configurations to facilitate isolation, processing, and amplification of a nucleic acid sample, as described in three exemplary embodiments below:

A first embodiment, as shown in FIG. 1C, of a fluidic 20 pathway 165 preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a shared fluid port 118, a sample segment 175 coupled to a sample port 114 and to the initial segment 174, and an s-shaped capture segment 166, configured to pass through the heating region 25 195 and a magnetic field 156, coupled to the initial segment 174 and the sample segment 175. In a variation of the first embodiment, the s-shaped capture segment 166 may comprise an initial wide arc 166 to provide a greater surface area for magnetic bead capture. In another variation of the first 30 embodiment, the capture segment 166 may alternatively be a progressively narrowing s-shaped capture segment 166. The first embodiment of the fluidic pathway 165 also comprises a reagent segment 176 coupled to a reagent port 115 and to the capture segment 166, a vent segment 177 coupled to the 35 reagent segment 176 and configured to pass through the vent region 190, a segment running to a detection chamber 163 from the vent region 190, a winding segment running away from the detection chamber 164, and an end vent 199 coupled to the segment running away from the detection chamber 164. 40 The first embodiment of the fluidic pathway 165 also comprises a first waste segment 178 configured to couple the initial segment 174 to the waste chamber 130, and a second waste segment 179 configured to couple the capture segment 166 to the waste chamber 130. The first waste segment 178 preferably functions to allow evacuation of excess release fluids from a fluidic pathway 165, for precise metering of the amount of release reagents used in a molecular diagnostic procedure using a low volume of sample.

In the first embodiment, the set of occlusion positions 141 50 comprises a first occlusion position 142 located along the initial segment 174 between points at which the initial segment couples to the fluid channel 119 and to the capture segment 166. The set of occlusion positions 141 also comprises a second occlusion position 143 located along the 55 sample segment 175, a third occlusion position 144 located along the reagent segment 176, a fourth occlusion position 145 located along the first waste segment 178, and a fifth occlusion position 146 located along the second waste segment 179. In the first embodiment, the set of occlusion posi- 60 tions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running 65 away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion posi16

tions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG.

The occlusion positions of the set of occlusion positions 141 of the first embodiment are preferably located such that occluding subsets of the set of occlusion positions 141 defines unique truncated fluidic pathways to controllably direct fluid flow. For example, as shown in FIG. 1D, occluding the fluidic pathway 165 at the first, third, fourth, and sixth occlusion positions 142, 144, 145, 147 forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port 114, may flow past the second occlusion positions 143 into the capture segment 166 for isolation and purification of nucleic acids using the heating region 195 and the magnetic field 156. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment 166 by the magnetic field 156, while other substances in the volume of sample fluid may pass into the waste chamber 130 by passing the fifth occlusion position 146. Following this subset of occlusion positions, the occlusion at the first occlusion position 142 may be reversed, as shown in FIG. 1E, and the fluidic pathway 165 may be occluded at the second occlusion position 143 to form a second truncated pathway by which a wash fluid may be delivered through the fluid port 118, into the capture segment 166 (thus washing the trapped magnetic beads), and into the waste chamber 130 by passing the fifth occlusion position 146. The occlusion at the second occlusion position 143 may then be reversed, and the first occlusion position 142 may be occluded (as shown in FIG. 1D), so that other fluidic pathways in the set of fluidic pathways 160 may be washed. After all fluidic pathways have been washed, a volume of air may be transferred through the fluid port 118 to prevent mixture of a wash solution with a release solution.

Thereafter in the first embodiment, as shown in FIG. 1E, the fluidic pathway 165 may be occluded at the second occlusion position 143 and the occlusion at the first occlusion 142 may be reversed, thus creating a third truncated pathway as shown in FIG. 1D. A release solution may then be delivered through the fluid port 118, into the capture segment 166, and to the waste chamber 130 by passing the fifth occlusion position 146. The release solution may then be sealed within a fourth truncated pathway (including the capture segment 166) of the fluidic pathway 165 by occluding the fluidic pathway at the fifth occlusion position 146, as shown in FIG. 1F. A release solution may then be delivered to other fluidic pathways of the set of fluidic pathways 160.

Thereafter, as shown in FIG. 1G, the occlusion at the fourth occlusion position 145 may be reversed, creating a fifth truncated pathway, and release solution within the fluidic pathway 165 may be metered by pumping air through the fluid port 118, which functions to push a portion of the release solution into the waste chamber 130. A volume of release solution will still be maintained within the capture segment 166 at this stage. As shown in FIG. 1H, the first and the fourth occlusion positions 142, 145 may then be occluded to form a sixth truncated pathway sealing the volume of release solution, with the captured magnetic beads bound to nucleic acids, within the capture segment 166. The volume of the remaining release solution is therefore substantially defined by the microchannel volume between junctions in the fluidic pathway 165 near the fourth and sixth occlusion positions 145, 147, and may be any small volume but in a specific variation is precisely metered to be 23+/-1 microliters. Release solution may be sealed within capture segments of other fluidic pathways using a similar process. A heater may

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then be provided at the sixth truncated pathway, inducing a pH shift within the sixth truncated pathway to unbind nucleic acids from the magnetic beads.

Thereafter in the first embodiment, as shown in FIG. 1I, the occlusions at the first and third occlusion positions 142, 144 5 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. ~20 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion 20 position (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 25 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detec- 30 tion chamber 117.

An alternative variation of the first embodiment may further comprise additional occlusion positions or alternative variations of the set of occlusion positions 141, such that occlusion at the additional occlusion positions permanently 35 seals the waste chamber from the fluidic pathway 165. Other alternative variations of the first embodiment may also comprise configurations of the set of occlusion positions 141 that are different than that described above. The variations may be configured, such that the a fluidic pathway 165 facilitates 40 meter release, does not allow meter release, facilitates addition of other reagents (e.g. neutralization or DNase reagents), facilitates additional washing steps, and/or facilitates other operations without changing the layout of the fluidic pathway **165** of a microfluidic cartridge embodiment. Thus, multiple 45 unique operations may be performed using the same microfluidic cartridge, by occluding fluidic pathways 160 at varied subsets of a set of occlusion positions 141.

A second embodiment, as shown in FIG. 6C, of a fluidic pathway 165' preferably comprises an initial segment 174' 50 fluidically coupled to a fluid channel 119' coupled to a shared fluid port 118', a sample segment 175' coupled to a sample port 114' and to the initial segment 174', and a capture segment 166', configured to pass through the heating region 195 and a magnetic field 156, coupled to the initial segment 174'. 55 The second embodiment of the fluidic pathway 165' also comprises a reagent segment 176' coupled to a reagent port 115' and to the turnabout portion 176', a vent segment 177' coupled to the reagent segment 176' and to the capture segment 166' and configured to pass through the vent region 190, 60 a segment running to a detection chamber 163' from the vent region 190, a segment running away from the detection chamber 164', and an end vent 199 coupled to the segment running away from the detection chamber 164'. The second embodiment of the fluidic pathway 165' also comprises a first waste 65 segment 178', coupled to the initial segment 174' at a point between points connecting the initial segment 174' to the

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sample segment 175' and to the capture segment 166'. The first waste segment 178' is configured to couple the initial segment 174' to the waste chamber 130. The second embodiment of the fluidic pathway 165' also comprises a second waste segment 179' configured to couple the capture segment 166' to the waste chamber 130', and an end vent segment 197' coupled to the capture segment 166' downstream of the point of connection to the second waste segment 179', and coupled to an end vent 199. The end vent segment 197' functions to provide fine metering of a fluid flowing through the fluidic pathway 165'.

In the second embodiment, the set of occlusion positions 141' comprises a first occlusion position 142' located along the initial segment 174' between points at which the initial segment couples to the fluid channel 119' and to the sample segment 175'. The set of occlusion positions 141' also comprises a second occlusion position 143' located along the sample segment 175', a third occlusion position 144' located along the reagent segment 176', a fourth occlusion position 145' located along the first waste segment 178', and a fifth occlusion position 146' located along the second waste segment 179'. In the second embodiment, the set of occlusion positions 141' also comprises a sixth occlusion position 147' located along the vent segment 177' upstream of the vent region 190, a seventh occlusion position 148' located along the segment running to the detection chamber 163', and an eighth occlusion position 149' located along the segment running away from the detection chamber 164'. Additionally, in the second embodiment, the set of occlusion positions 141 comprises a ninth occlusion position 157' located along the sample segment 175' between the sample port 114 and the second occlusion position 143, a tenth occlusion position 158' located along the end vent segment 197, and an eleventh occlusion position 159' located along the capture segment 166' between points at which the capture segment 166' couples to the end vent segment 197' and to the vent segment 177'.

The occlusion positions of the set of occlusion positions 141' of the second embodiment are preferably located such that occluding of subsets of the set of occlusion positions 141' defines unique truncated fluidic pathways to controllably direct fluid flow. For example, occluding the fluidic pathway 165' at the first, fourth, sixth, tenth, and eleventh occlusion positions 142', 145', 147', 158', 159' forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port 114, may flow into the capture segment 166' for isolation and purification of nucleic acids using the heating region 195 and the magnetic field 156. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment 166' by the magnetic field 156, while other substances in the volume of sample fluid may pass into the waste chamber 130 by passing the fifth occlusion position 146'. Following this subset of occlusion positions, the occlusion at the first occlusion position 142' may be reversed, and the fluidic pathway 165' may be occluded at the second occlusion position 143' to form a second truncated pathway by which a wash fluid may be delivered through the fluid port 118, into the capture segment 166' (thus washing the trapped magnetic beads), and into the waste chamber 130 by passing the fifth occlusion position 146'. A volume of air may then be pumped through the fluid port 118 to flush any remaining wash solution into the waste chamber 130

Thereafter, in the second embodiment, the fluidic pathway 165' may be occluded at the fifth occlusion position 146' and the occlusion at the tenth occlusion position 158' may be reversed, closing access to the waste chamber 130 and open-

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ing access to the end vent segment 197'. A release solution may then be delivered through the fluid port 118, into the capture segment 166', and to the end vent segment 197'. The volume of the release solution is therefore defined by the microchannel volume between the fourth and tenth occlusion 5 positions 145', 158', and may be any small volume but in a specific variation is precisely metered to be 15 microliters. Thereafter, occluding the fluidic pathway 165' at the tenth occlusion position 158', reversing the occlusion at the fourth occlusion position 145' (defining a fourth truncated pathway), and delivering air through the fluid port 118 pushes any remaining release buffer from the fluidic pathway 118 into the waste chamber 130, thereby ensuring that excess release buffer is not later exposed to nucleic acids bound to the magnetic beads (at this point, the nucleic acids are not sub- 15 stantially released from the magnetic beads because heat has not been added). Thereafter, the fluidic pathway 165' is occluded at the first and fourth occlusion positions 142', 145', defining a fifth truncated pathway comprising the capture segment 166', and the magnetic beads are heated to an appro- 20 priate temperature and time (e.g., 60 degrees for 5 minutes) within the heating region 195 to release the nucleic acids from the magnetic beads and into the release buffer.

Thereafter, in the second embodiment, the occlusions at the first and eleventh occlusion positions 142', 159' are reversed, 25 defining a sixth truncated pathway, the entire released nucleic acid sample (e.g. ~15 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent mixture stored off of the microfluidic cartridge 100. During the reconstitution process, the occlusion at the sixth occlusion position 147' may be reversed, thus defining a seventh truncated pathway. Once reconstitution of the molecular diagnostic reagent mixture with the released nucleic acid sample is complete and well 35 mixed, the reconstituted mixture may then be aspirated through the reagent port 115 through the seventh truncated pathway to the detection chamber 117, completely filling the detection chamber 117, after which the fluidic pathway 165' is be occluded at third, seventh, eighth, and ninth occlusion 40 positions 144', 148', 149', 157' defining an eighth truncated pathway. An external molecular diagnostic system and/or module may then perform additional processes on the volume of fluid within the detection chamber 117.

An alternative variation of the second embodiment may 45 further comprise additional occlusion positions or alternative variations of the set of occlusion positions 141', such that occlusion at the additional occlusion positions permanently seals the waste chamber from the fluidic pathway 165'. Other alternative variations of the second embodiment may also 50 comprise configurations of the set of occlusion positions 141' that are different than that described above.

A third embodiment, as shown in FIG. 7, of a fluidic pathway 165" preferably comprises an initial segment 174" fluidically coupled to a fluid channel 119" coupled to a shared 55 fluid port 118, a sample segment 175" coupled to a sample port 114 and to the initial segment 174", and a capture segment 166" coupled to the initial segment 174". The third embodiment of the fluidic pathway 165" also comprises a reagent segment 176" coupled to a reagent port 115, a vent segment 177" coupled to the reagent segment 176" and to the capture segment 166", and configured to pass through the vent region 190, a segment running to a detection chamber 163" from the vent region 190, a segment running away from the detection chamber 164", and an end vent 199 coupled to 65 the segment running away from the detection chamber 164". The third embodiment of the fluidic pathway 165" also com-

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prises a first waste segment 178" configured to couple the initial segment 174" to the waste chamber 130, and a second waste segment 179" configured to couple the capture segment 166" to the waste chamber 130.

In the third embodiment, the set of occlusion positions 141" comprises a first occlusion position 142" located along the initial segment 174" between points at which the initial segment 174" couples to the fluid channel 119" and to the sample segment 175". The set of occlusion positions 141" also comprises a second occlusion position 143" located along the sample segment 175", a third occlusion position 144" located along the reagent segment 176", a fourth occlusion position 145" located along the first waste segment 178", and a fifth occlusion position 146" located along the second waste segment 179". In the third embodiment, the set of occlusion positions 141" also comprises a sixth occlusion position 147" located along the vent segment 177" upstream of the vent region 190, a seventh occlusion position 148" located along the segment running to the detection chamber 163", an eighth occlusion position 149" located along the segment running away from the detection chamber 164", and a ninth occlusion position 157" located along the vent segment 177" between the point at which the vent segment 177" couples to the second waste segment 179" and the sixth occlusion point 147".

Similar to the first and the second embodiments, the occlusion positions of the set of occlusion positions 141" of the third embodiment are preferably located such that an occlusion of subsets of the set of occlusion positions 141" defines unique truncated fluidic pathways to controllably direct fluid flow. Example truncated fluidic pathways, defined by occluding the fluidic pathway 165" using subsets of the set of occlusion positions 141", are shown in FIG. 7.

Preferably, a fluidic pathway 165 of the set of fluidic pathways 160 comprises at least one of a first channel type 171, a second channel type 172 with a reduced cross sectional area, and a third channel type 173 with an curved surface as shown in FIG. 8A. A variation of the first channel type 171 has an approximately rectangular cross section with slightly sloping walls, such that at least two walls of the first channel type 171 slope toward each other to facilitate manufacturing of the first channel type 171; however, alternative variations of the first channel type 171 may have non-sloping walls or walls that slope away from each other. In specific embodiments of the first channel type 171, the walls of the first channel type 171 slope at 6° from vertical, to facilitate extraction of injection molded parts, and are between 300 and 1600 microns wide and between 100 and 475 microns tall. In a first specific embodiment of the second channel type 172, the cross section of the second channel type 172 is a 250 micron wide equilateral triangle with the top truncated to be 200 microns deep. In a second specific embodiment of the second channel type 172, the cross section of the second channel type is a truncated triangle that is 160 microns wide and 160 microns deep. In a specific embodiment of the third channel type 173, the surface of the third channel type is defined by Gaussian function, and is 800 microns wide and 320 microns deep. Alternative embodiments of the third channel type 173 may comprise a surface defined by any appropriate curved function.

The first channel type 171 is preferably used over a majority of a fluidic pathway 165, and preferably in portions near a vent region 190, in a capture segment 166 configured to pass through a magnetic field 156, and in a segment leading to a Detection chamber 163. Preferably, an embodiment of the first channel type 171, comprising a wide channel with little depth is used in regions configured to pass through a magnetic field 156, such that particles in the regions are driven closer to

the magnetic field source. The second channel type 172 is preferably used near a vent region 190 of a fluidic pathway 165, and preferably in portions of a fluidic pathway 165 leading to and away from a detection chamber 163, 164 (to constrict fluid flow into the Detection chamber 117). The third 5 channel type 173 is preferably used in a portion of a fluidic pathway 165 near a normally open position 42 of the set of occlusion positions 141. Transitions between different channel types 171, 172, 173 may be abrupt, or alternatively, may be gradual, as shown in FIG. 8B. The first, second, and third 10 channel types 171, 172, 173 may also alternatively be used in any appropriate portion of a fluidic pathway 165. Example embodiments of channel types for segments of a fluidic path-

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way are shown in FIG. 8C Multiple fluidic pathways may be configured to pass 15 through a single heating region 195 of the microfluidic cartridge 100, a single vent region 190 of the microfluidic cartridge 100, and/or a magnetic field 156 produced by a magnet 152 housed within a single magnet housing region 150. Preferably all fluidic pathways of the set of fluidic pathways 160 20 are configured to pass through a single heating region 195 of the microfluidic cartridge 100, a single vent region 190 of the microfluidic cartridge 100, and a magnetic field 156 produced by a magnet 152 housed within a single magnet housing region 150; however, alternative embodiments of the set of 25 fluidic pathways 160 of the microfluidic cartridge may comprise different configurations wherein fluidic pathways of the set of fluidic pathways 160 do not share a single heating region 195, a single vent region 190, and/or a magnetic field

Additionally, the set of fluidic pathways 160 of the microfluidic cartridge 100 may comprise virtually any number of fluidic pathway 165 and/or the set of Detection chambers 116 may comprise virtually any number of Detection chambers 116 as can practically be integrated into the microfluidic 35 cartridge 100. In one specific embodiment, the set of fluidic pathways 160 may comprise twelve fluidic pathways 165, four of which are shown in FIG. 9.

1.6 Microfluidic Cartridge—Additional Microfluidic Cartridge Elements

The microfluidic cartridge 100 is preferably configured such that actual valving members are not integrated into the microfluidic cartridge 100, thus, opening and/or occluding portions of a fluidic pathway 165 are performed by systems located external to the microfluidic cartridge. As an example, 45 portions of a fluidic pathway 165 may be opened or occluded at occlusion positions, as described above, by the action of a valving member or mechanism held beneath the card that applies a biasing force to deform the elastomeric layer 140 and occlude a fluidic pathway 165. The force may be applied 50 by a mechanical member (e.g., a pin, post, etc.), an electromechanical member (e.g. a solenoid), a pneumatic or hydraulic member (e.g., air, water, etc.) or any other appropriate means, as shown in FIGS. 10A and 10B. In some variations, the cartridge may include one or more registration regions 55 that allow the card to be aligned with respect to the valving member or mechanism. In alternative embodiments, the elastomeric layer 140, the set of valve guides 127, and the set of occlusion positions 141 may be omitted and replaced with valves integrated within the microfluidic cartridge 100, that 60 are configured to controllably occlude and open portions of a fluidic pathway 165.

Other embodiments of the microfluidic cartridge 100 may further comprise a tag 198 that functions to encode and provide identifying information related to the microfluidic cartridge 100. The tag 198 may comprise a barcode, QR code, or other optical machine-readable tag, or may alternatively be an

electronic tag, such as an RFID chip. The identifying information preferably comprises at least information relating to the position of a microfluidic cartridge 100 within a molecular diagnostic system, and information relating to samples analyzed using the microfluidic cartridge 100 (e.g. how many positions remain available for conducting tests). In alternative variations, the tag may relate other information about samples (e.g. sample type, sample volume, sample concentration, date) processed using the microfluidic cartridge 100. Preferably, the tag does not interfere with procedures being performed using the microfluidic cartridge, and is located in an unobtrusive position on the microfluidic cartridge 100, such as a side panel of the microfluidic cartridge 100. Alternatively, the microfluidic cartridge 100 may not comprise a tag 198, and a user or other entity may relate identifying information to the microfluidic cartridge 100 using any appropri-

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As a person skilled in the art will recognize from the previous detailed description and from the FIGURES and claims, modifications and changes can be made to the preferred embodiments of the microfluidic cartridge 100 without departing from the scope of this invention, as is shown in the example embodiment shown in FIGS. 11A and 11B, and in the alternative example embodiment of FIGS. 6A-6C, wherein in the orientation of FIG. 6B, the intermediate substrate 120 comprising a waste chamber 130 is coupled to the top layer 110, and the elastomeric layer 140 is located on the bottom of the microfluidic cartridge 100.

2. Specific Embodiment of a Microfluidic Cartridge

The following description a specific embodiment of the microfluidic cartridge 100 is for illustrative purposes only, and should not be construed as definitive or limiting of the scope of the claimed invention.

The specific embodiment of the microfluidic cartridge 100, as shown in FIGS. 11A and 11B, meets SLAS ANSI guidelines for a microtiter plate footprint, governing the dimensions of the specific embodiment of the microfluidic cartridge 100. The specific embodiment of the microfluidic cartridge 100 is thus 127.76 mm long and 850.48 mm wide.

The specific embodiment of the microfluidic cartridge 100 comprises a top layer no including a set of twelve sample port-reagent port pairs 112, a set of twelve Detection chambers 116, a shared fluid port 118, a heating region 195, and a vent region 190, an intermediate substrate 120, coupled to the top layer no and partially separated from the top layer no by a film layer 125, configured to form a waste chamber 130, an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber, and a set of fluidic pathways 160, formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140.

The top layer 110 of the specific embodiment of the microfluidic cartridge 100 functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR. The majority of the top layer 110 of the specific embodiment is 1.5 mm thick (aside from regions defining ports, the vent, the heating region 195 or fluidic pathways 165), and is produced by injection molding without the use of a mold release. The polypropylene is clear to allow transmission of light in the detection chambers. The injection molding process defines the set of 12 sample port-reagent port pairs, which are located along one long edge of the top layer 110, and also defines the set of 12 detection chambers 116, which are located along the opposite long edge of the top layer 110.

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The Detection chambers 117 do not completely transect the top layer 110, as shown in FIGS. 11A and 11B. Each detection chamber 117 of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of ~10 mL for each detection chamber 117. The dimensions of the detection chambers 117 of the specific embodiment are such that the detection chambers 117 facilitate heating from one side (resulting in simpler heater design yet fast cycling given the small depth of the channels), and also facilitate the injection molding process. The bottoms of the detection chambers 117 are formed by the film layer 125, which is polypropylene film 15 compatible with PCR (100 microns thick or less) that offers low autofluorescence. The film layer 125 can withstand temperatures up to 120° C. or more.

The injection molding process also defines the shared fluid port 118 of the top layer 110, and the vent region 190, which 20 is recessed 0.5 mm into the top surface of the top layer 110 (in the orientation shown in FIG. 11B), and is covered with a polytetrafluoroethylene membrane, which is hydrophobic, gas permeable, and liquid impermeable. A paper label is bonded with adhesive to the top layer 110 over the vent region 25 190, which serves to identify the cartridge and protect the vent region 190, as shown in FIGS. 11A and 11B. The injection molding process also defines the heating region 195, which is recessed and spans the long dimension of the top layer 110. slightly offset from a midline of the top layer 110. The top 30 layer 110 of the specific embodiment requires approximately 15 grams of polypropylene, and all draft angles for the top layer 110 are a minimum of 4 degrees, as defined by the injection molding process.

In the specific embodiment, the intermediate substrate 120 35 is composed of a polypropylene material to minimize cost and simplify assembly, and in the orientation shown in FIG. 11B, the top of the intermediate substrate 120 is 1.5 mm thick. The film layer 125, partially separating the intermediate substrate nominal thickness of 50 microns. The film layer 125 is able to withstand temperatures of up to 95° C. encountered during fabrication and during an intended PCR procedure, while being thermally bondable to the top layer 110. The top layer 110 and the film layer 125 are bonded using thermal fusion 45 bonding, and this subassembly is bonded to the intermediate substrate 120 using a polymer adhesive. Additionally, for aligning layers 110, 120, 125 and bonding the top layer 110 to the intermediate substrate 120, plastic studs are configured to extend from the top of the intermediate substrate 120 through 50 die-cut holes in the film layer 125 and injection molded holes in the bottom of the top layer 110. The intermediate substrate also comprises a set of valve guides 127, at a set of occlusion positions 141, which are holes with chamfered edges through the intermediate substrate 127. Each valve guide in the set of 55 valve guides 127 is 2.1 mm×2.1 mm square, and configured to accommodate an occluder with a 2 mm×2 mm square head for normally open positions 42 or 2.1 mm diameter circle to accommodate a 2 mm diameter round pin for normally closed positions 43.

The elastomeric layer 140 of the specific embodiment is composed of a low durometer silicone, and comprises strips that are 500 microns thick and that can withstand temperatures of 120° C. at a minimum. The strips of the elastomeric layer are arranged over the set of valve guides 127, and 65 bonded to the top of the intermediate substrate 120 using a silicone adhesive. Additionally, the elastomeric layer 140 is

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slightly compressed between the film layer 125 and the top of the intermediate substrate (in the orientation shown in FIG. 11B).

The bottom layer 170 of the specific embodiment of the microfluidic cartridge 100 is composed of polypropylene, identical to that of the intermediate substrate 120. The bottom layer is 1.5 mm thick, and is contiguous in the area of the set of Detection chambers 116, such that an outer perimeter of the entire bottom layer 170 substantially spans the footprint of the microfluidic cartridge 100. The bottom layer 170 of the specific embodiment is bonded to the intermediate substrate 120 using polymer adhesive, providing a hermetic seal that ensures that a waste fluid within the waste chamber 130 of the intermediate substrate 120 does not leak out of the waste chamber 130.

The specific embodiment of the microfluidic cartridge 100 comprises twelve fluidic pathways 165 in the set of fluidic pathways 160, such that the microfluidic cartridge 100 is capable of testing up to twelve samples using twelve distinct fluidic pathways 165. Each of the twelve fluidic pathways 165 is coupled to one of the twelve sample port-reagent port pairs 113 on one end of the microfluidic cartridge 100, and coupled to one of the twelve detection chambers 117 on the other end of the microfluidic cartridge, as shown in FIGS. 11A and 11B. Each fluidic pathway 165 is substantially identical (aside from portions connecting to an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118) and identical to the first embodiment of a fluidic pathway described in Section 1.5 and shown in FIG. 1C. Additionally, the microfluidic channels comprising each fluidic pathway 165 are of the first channel type 171 and 500 microns wide by 475 microns deep, aside from the microfluidic channels of the segments leading to and away from the detection chambers 163, 164, the turnabout portions 166, and the vent segments 177. Also, parallel microfluidic channels of the fluidic pathways 165 of the specific embodiment are typically evenly spaced at 2.25 mm (center-to-center).

The fluidic pathways 165 of the specific embodiment are, 120 from the top layer 110 is a polypropylene film with a 40 in their default condition, open at all occlusion positions, aside from the fourth, seventh, and eighth, occlusion positions 145, 148, 149, as shown in FIG. 1C. Furthermore, the s-shaped capture segment 166 of a fluidic pathway of the specific embodiment is configured to have a volume capacity of 22  $\mu$ L, have a width of 5.5 mm, and weave back and forth over a magnetic field 156, by crossing the magnet housing region 150. The depth of the s-shaped capture segment 166 is 0.4 mm for the 1.6 mm wide channels and 0.475 for the 0.5 mm narrower channel.

> The specific embodiment also comprises a barcode tag 198 located on a vertical edge of the microfluidic cartridge 100, as shown in FIG. 11A. Additional features of the specific embodiment of the microfluidic cartridge 100 are shown in FIGS. 11A and 11B.

3. Assembly Method for an Embodiment of the Microfluidic An embodiment of an assembly method 200 for an embodiment of the microfluidic cartridge 100 is shown in FIGS. 12A-12G. The assembly method 200 preferably comprises aligning the top layer to the film layer and thermally 60 bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge S210; compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate S220; bonding the intermediate substrate to the bottom layer S230; installing the vents of the vent region S250; and applying labels and packaging S260.

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Step S210 recites aligning the top layer to the film layer and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge, and functions to create a first subassembly comprising the top layer, the film layer, the elastomeric layer, and the intermediate substrate. Preferably, the elastomeric layer is glued with silicone to the intermediate substrate; however, the elastomeric layer may alternatively be solely compressed between the top layer/film layer and the intermediate substrate, without any adhesive. Preferably, a 10 first jig is used to align the top layer and the film layer using pins in the jig and holes in the layers, and in an example embodiment of S210, the top layer is first placed face down in the first jig, and the film layer is placed onto the top layer in preparation for thermal bonding using a lamination machine 15 or hot press. In the example embodiment of S210, the elastomeric layer is then fit over ultrasonic welding tabs in of the top layer, as shown in FIGS. 12D and 12F, however, processes other than ultrasonic welding may be used. An adhesive may also be applied around the border of the elastomeric layer, to 20 prevent leakage between the elastomeric layer and the intermediate substrate. Protrusions molded into the top of the intermediate substrate are then passed through alignment holes in the top layer, thus aligning the top layer, the elastomeric layer, and the intermediate substrate of the microfluidic 25 cartridge. In alternative embodiments of S210, any appropriate alignment mechanism may be used to align the top layer, the elastomeric layer, and the intermediate substrate, using for example, a combination of adhesives, frames, and alignment pins/recesses.

Step S220 recites compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate, and functions to seal the layers in order to prevent leakage between the layers. Preferably, S220 forms 35 detecting nucleic acid in a sample, comprising: hermetic seals between the top layer and the elastomeric layer, and the elastomeric layer and the intermediate substrate, in embodiments of S210 where an adhesive application is involved. In an example embodiment of S220, the first jig with the top layer, the elastomeric layer, and the intermediate 40 substrate is placed within an ultrasonic welder to be compressed and ultrasonically welded.

Step S230 recites bonding the intermediate substrate to the bottom layer S230, which functions to form a second subassembly comprising the top layer, the elastomeric layer, the 45 intermediate substrate, and the bottom layer. Preferably, the bottom layer self-aligns with the intermediate substrate as a result of the bottom layer fitting completely inside a recessed flange on the lower portion of the intermediate layer. The bottom layer is preferably thermally bonded to the intermediate layer. Alternatively, the bottom layer may be bonded to the intermediate layer using adhesive or ultrasonic welding, as shown in FIG. 12G.

Step S250 recites installing the vents of the vent region S250, which functions to permanently form the vents of the 55 vent region. Step S250 is preferably performed by heat staking the vents in place, but may alternatively be performed using adhesive or solvent bonding process. Following step S250, the assembly method 200 may further comprise certain quality control measures, including pressure testing the 60 microfluidic cartridge S252 by blocking all sample and reagent ports, and injecting air into the fluid port, and removing the finished microfluidic cartridge from the second jig S254. Step S260 recites applying labels and packaging, and functions to prepare the microfluidic cartridge with identify- 65 ing information using at least a barcode label, and preparing the microfluidic cartridge for commercial sale.

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An alternative embodiment of an assembly method 300, as shown in FIG. 13, comprises thermally bonding the film layer to the top layer to form a first subassembly S310; adding a vent to the first subassembly and applying a label to create a second subassembly S320; applying an adhesive inside a bottom flange of the intermediate substrate and bonding the bottom layer to the intermediate substrate S330; applying a tag to the intermediate substrate to create a third subassembly S340; positioning the elastomeric layer on the third subassembly to create a fourth subassembly S350; applying adhesive to the fourth subassembly S360; and coupling the second subassembly to the fourth subassembly S370.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of methods according to preferred embodiments, example configurations, and variations thereof. It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose systems that perform the specified functions or acts.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

#### We claim:

- 1. A cartridge, configured to facilitate processing and
  - a first layer comprising a sample port and a fluid port;
  - an intermediate substrate coupled to the first layer and defining a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the waste chamber and accessible from a direction perpendicular to a broad surface of the first layer; and
  - a fluidic pathway, superior to the intermediate substrate, at least partially separated from the corrugated surface of the waste chamber by an elastomeric laver, and formed by at least a portion of the first layer, wherein the fluidic pathway is fluidically coupled to the sample port and the fluid port, wherein the fluidic pathway is configured to be occluded at a set of occlusion positions through at least one void of the set of voids of the corrugated surface by deformation of the elastomeric layer, and wherein the fluidic pathway is configured to transfer waste fluid to an interior portion of the corrugated surface of the waste chamber, through a set of openings of the intermediate substrate, upon occlusion of a subset of the set of occlusion positions.
- 2. The cartridge of claim 1, wherein the first layer is a unitary construction comprising a reagent port, the fluid port, and a detection chamber, and wherein the fluidic pathway is coupled to the reagent port, the fluid port, and the detection
- 3. The cartridge of claim 2, wherein the sample port and the reagent port are configured to couple to a standard pipette tip, and the fluid port is configured to couple to a syringe pump.
- 4. The cartridge of claim 2, wherein the first layer further comprises a vent region, and wherein the fluidic pathway is configured to pass through the vent region upstream of the

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detection chamber, and wherein the vent region comprises a hydrophobic, liquid-impermeable membrane that is gas-permeable

- 5. The cartridge of claim 1, wherein the waste chamber comprises at least one waste inlet and a waste vent, and 5 wherein at least one void of the set of voids defined by the corrugated surface of the waste chamber is a rectangular prismatic void spanning a long dimension of the cartridge that defines a magnet housing region configured to reversibly receive a magnet from the direction perpendicular to the 10 broad face of the first layer, and configured to cross the fluidic pathway.
- 6. The cartridge of claim 5, wherein the fluidic pathway comprises an s-shaped capture segment configured to cross the magnet housing region multiple times.
- 7. The cartridge of claim 6, wherein the fluidic pathway is configured to transfer a waste fluid to the waste chamber through a first waste inlet upstream of the capture segment by way of a first waste segment fluidly coupled to the first waste inlet and an initiating portion of the capture segment, and wherein the fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet downstream of the capture segment by way of a second waste segment fluidly coupled to the second waste inlet and a terminating portion of the capture segment.
- 8. The cartridge of claim 1, wherein the elastomeric layer is situated between the fluidic pathway of the first layer and the intermediate substrate, wherein the intermediate substrate includes a set of openings defining a set of occlusion positions, accessible at least at a subset of the set of voids of the 30 corrugated surface of the waste chamber, providing access to the elastomeric layer, such that the fluidic pathway is configured to be occluded through the elastomeric layer at the set of occlusion positions from a direction perpendicular to the broad surface of the first layer.
- 9. The cartridge of claim 8, wherein at least one void of the set of voids defined by the corrugated surface of the waste chamber is defined by five surfaces of the corrugated surface, including a first pair of parallel surfaces and a second pair of parallel surfaces orthogonal to the first pair of parallel surfaces and orthogonal to the broad surface of the first layer, and a surface, coupled to the first pair of surfaces and the second pair of surfaces and proximal to the first layer, having a set of openings for occlusion of the fluidic pathway at the set of occlusion positions, and wherein occluding the fluidic pathway at a first subset of the set of occlusion positions defines a truncated pathway configured to facilitate transfer of a waste fluid to the waste chamber.
- 10. A cartridge, configured to facilitate processing and detecting of nucleic acids, comprising:
  - a first layer and an intermediate substrate, coupled to the first layer, wherein the intermediate substrate defines a waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of the intermediate substrate and external to the waste chamber, wherein the set of voids is accessible from a direction perpendicular to a broad surface of the first layer:
  - a first fluidic pathway, formed by at least a portion of the 60 first layer; and
  - a second fluidic pathway in parallel with the first fluidic pathway, formed by at least a portion of the first layer, wherein the first fluidic pathway and the second fluidic pathway are each superior to the intermediate substrate, 65 are each at least partially separated from the corrugated surface of the intermediate substrate by an elastomeric

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layer and are each configured to transfer waste to the waste chamber through a set of openings of the intermediate substrate.

- 11. The cartridge of claim 10, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample portreagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber, wherein the first fluidic pathway is substantially identical to the second fluidic pathway, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.
- 12. The cartridge of claim 11, wherein the first detection chamber is thermally and optically isolated from the second detection chamber.
- 13. The cartridge of claim 11, further comprising a heating region as a recessed region of the first layer that is parallel to the set of parallel voids of the corrugated surface, and a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber.
- **14**. The cartridge of claim **13**, wherein the vent region comprises a liquid impermeable membrane.
- 15. The cartridge of claim 13, wherein at least of the first fluidic pathway and the second fluidic pathway is coupled to an end vent configured to provide fine metering of fluid flow.
- 16. The cartridge of claim 11, wherein each sample port and each reagent port in the first sample port-reagent port pair and the second sample port-reagent port pair is configured to couple to a pipette tip, and the fluid port is configured to couple to a syringe pump.
  - 17. The cartridge of claim 11, wherein the fluid port is a shared fluid port, configured to fluidically couple to the first fluidic pathway and the second fluidic pathway.
  - 18. The cartridge of claim 11, wherein the first detection chamber comprises a first serpentine-shaped fluidic channel, and the second detection chamber comprises a second serpentine-shaped fluidic channel.
  - 19. The cartridge of claim 18, wherein at least one of the first serpentine-shaped fluidic channel and the second serpentine-shaped fluidic channel comprises three wide channels directly interconnected by two narrow channels, wherein the three wide channels include a first wide channel comprising a detection chamber inlet into the detection chamber and a second wide channel comprising a detection chamber outlet from the detection chamber.
- ing the first layer, wherein the corrugated surface defines a set of parallel voids spanning a majority of a width of 55 chamber is configured to be heated from one side, and the second detection chamber is configured to be heated from one chamber, wherein the set of voids is accessible from a side.
  - 21. The cartridge of claim 11, wherein the first detection chamber and the second detection chamber are identical, and configured to be imaged using an optical subsystem.
  - 22. The cartridge of claim 11, wherein at least one of the first detection chamber and the second detection chamber is configured to be optimized for volumetric capacity, thermocycling rates, optical detection, and filling in a manner that limits bubble generation.
  - 23. The cartridge of claim 10, wherein at least one void of the set of voids defined by the corrugated surface of the waste

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chamber is a rectangular prismatic void spanning a long dimension of the cartridge defining a magnet housing region configured to cross the fluidic pathway.

24. The cartridge of claim 10, wherein the elastomeric layer is situated between the first layer and the intermediate substrate, wherein the intermediate substrate includes the set of openings defining a set of occlusion positions, accessible through at least at a subset of the set of voids of the corrugated surface of the waste chamber, providing access to the elastomeric layer, such that the first fluidic pathway and the second fluidic pathway are configured to be occluded through the elastomeric layer at the set of occlusion positions, and wherein the waste chamber is located inferior to the elastomeric layer.

25. The cartridge of claim 8, wherein the fluidic pathway is configured to transfer a waste fluid to the waste chamber through a first waste inlet upon occlusion of a first subset of the set of occlusion positions by way of a first waste segment fluidly coupled to the first waste inlet and a first portion of the fluidic pathway proximal the sample port, and wherein the fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet upon

occlusion of a second subset of the set of occlusion positions by way of a second waste segment fluidly coupled to the second waste inlet and a second portion of the fluidic pathway substantially downstream of the sample port.

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26. The cartridge of claim 24, wherein the first fluidic pathway is configured to transfer a first waste fluid to the waste chamber through a first waste inlet upon occlusion of a first subset of the set of occlusion positions by way of a first waste segment fluidly coupled to the first waste inlet inline with the first fluidic pathway, and wherein the second fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet upon occlusion of a second subset of the set of occlusion positions by way of a second waste segment fluidly coupled to the second waste inlet inline with the second fluidic pathway.

27. The cartridge of claim 1, wherein the intermediate substrate is partially separated from the first layer by a film layer.

28. The cartridge of claim 10, wherein the intermediate substrate is partially separated from the first layer by a film layer.

\* \* \* \* \*

# EXHIBIT 26

## (12) United States Patent

Williams et al.

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#### (54) SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

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	B01L 3/00	(2006.01)
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	C12M 3/00	(2006.01)
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	B29C 65/08	(2006.01)
	B29C 65/60	(2006.01)
	B29C 65/00	(2006.01)
	B29L 31/00	(2006.01)

(52) U.S. Cl.

CPC ...... *B01L 3/502* (2013.01); *B01L 3/5027* (2013.01); B01L 3/502707 (2013.01); B01L 3/502738 (2013.01); B01L 7/52 (2013.01); **B01L** 7/525 (2013.01); **C12M** 23/42 (2013.01); C12Q 1/68 (2013.01); C12Q 1/686 (2013.01); B01L 3/5025 (2013.01); B01L 3/50273 (2013.01); B01L 3/502723 (2013.01); B01L 3/502746 (2013.01); B01L 2200/0684 (2013.01); B01L 2200/0689 (2013.01); B01L 2200/10 (2013.01); B01L 2200/142 (2013.01); B01L 2300/021 (2013.01); B01L 2300/022 (2013.01); B01L 2300/087 (2013.01); B01L 2300/0809 (2013.01); B01L 2300/0816 (2013.01); B01L 2300/0861 (2013.01); B01L 2300/0867 (2013.01); B01L 2300/0883 (2013.01); B01L 2300/0887 (2013.01); B01L 2300/123 (2013.01); B01L 2300/14 (2013.01); B01L 2300/16 (2013.01); B01L 2300/1827 (2013.01); B01L 2400/043 (2013.01); B01L 2400/0406 (2013.01); B01L 2400/0481 (2013.01); B01L 2400/0487 (2013.01); B01L 2400/0622 (2013.01); B01L 2400/0655 (2013.01); B01L 2400/0694 (2013.01); B01L 2400/086 (2013.01); B29C 65/08 (2013.01); B29C 65/606 (2013.01); B29C 66/71 (2013.01); B29C 66/81423 (2013.01); B29C 66/8322 (2013.01); B29L 2031/756 (2013.01); C12N 13/00 (2013.01)

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#### (57) ABSTRACT

A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.

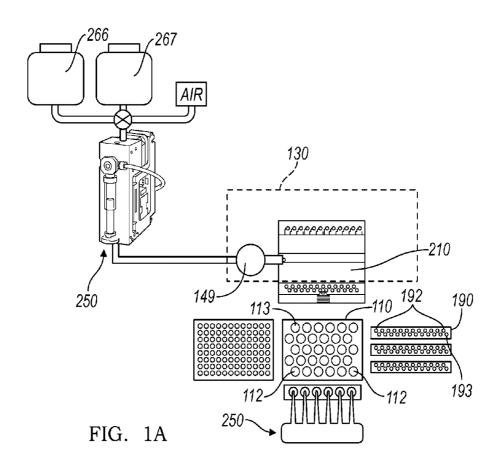
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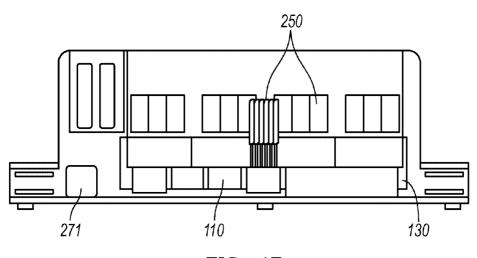


FIG. 1B

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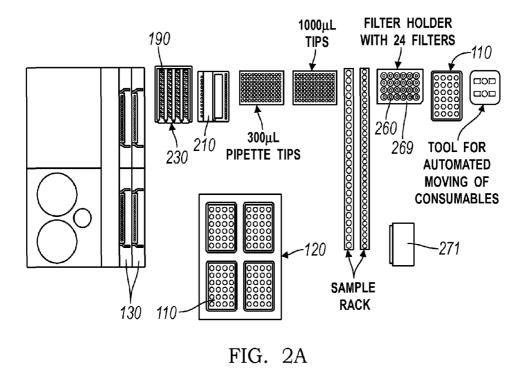


FIG. 2B

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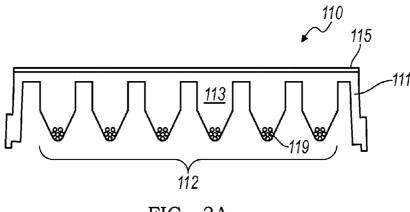
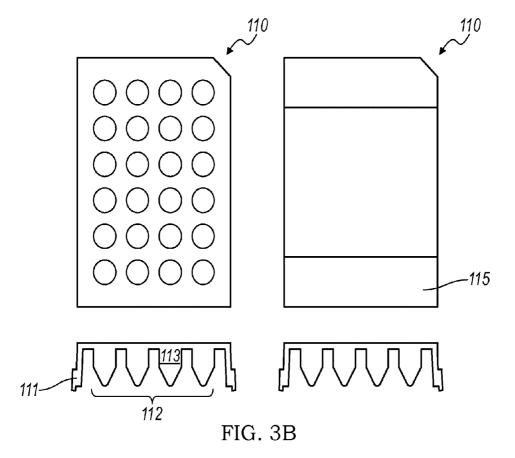


FIG. 3A



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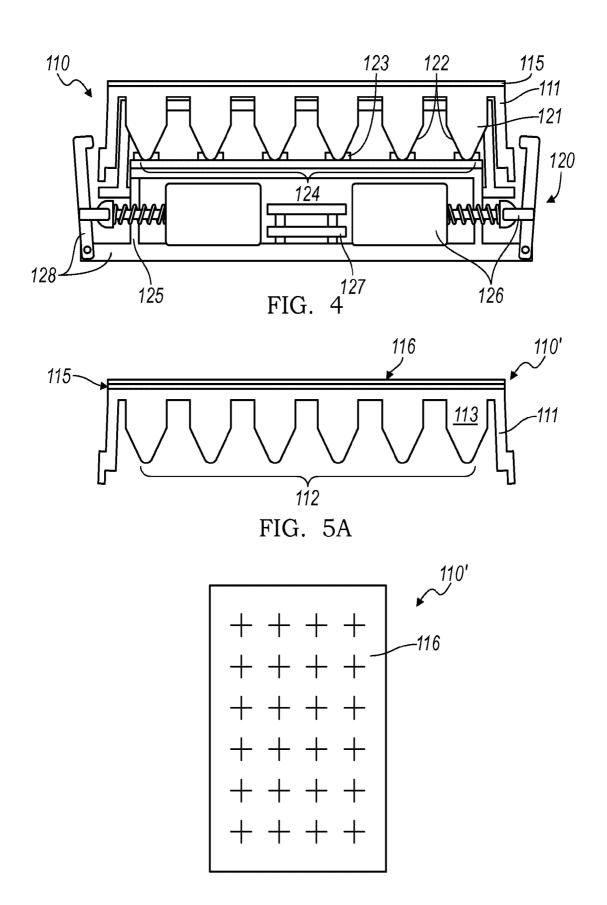
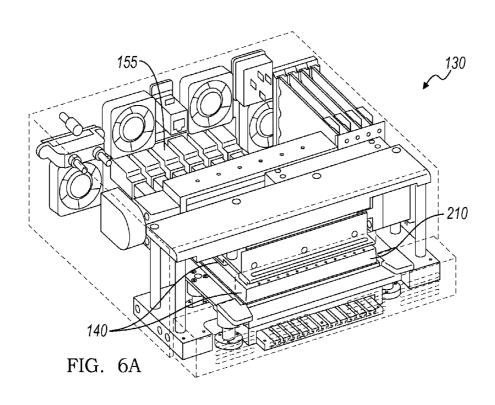
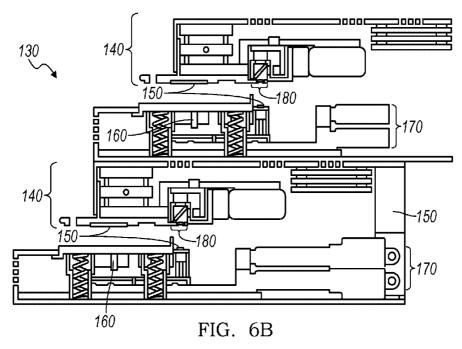


FIG. 5B

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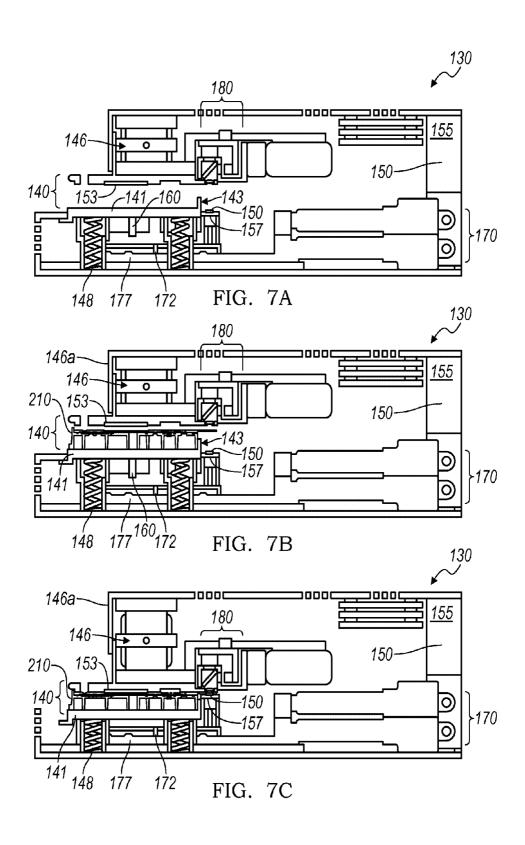
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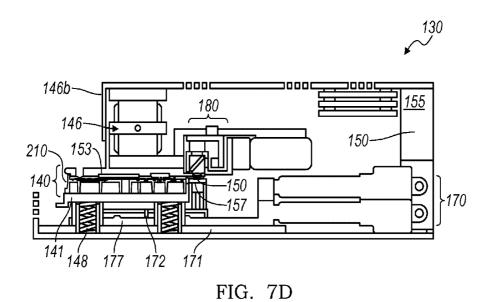
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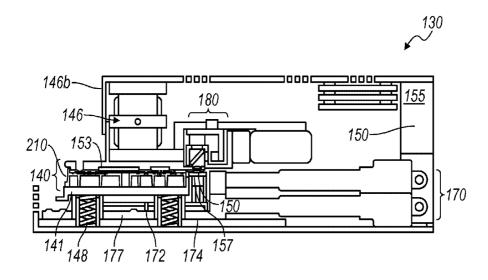
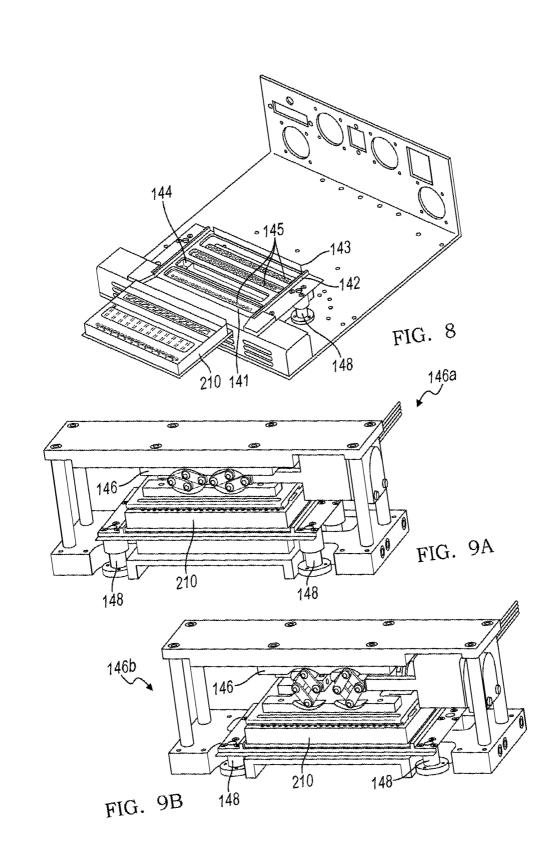
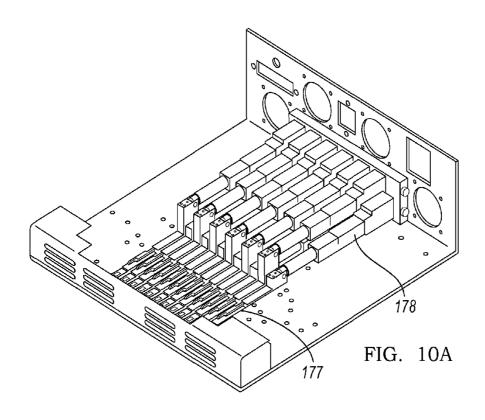


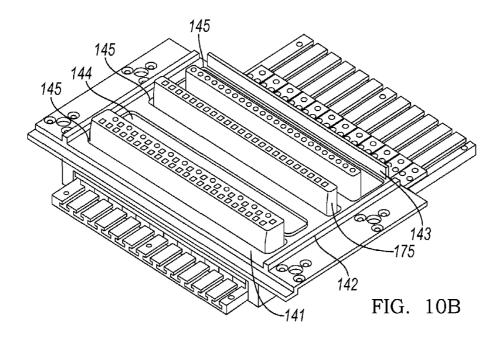
FIG. 7E



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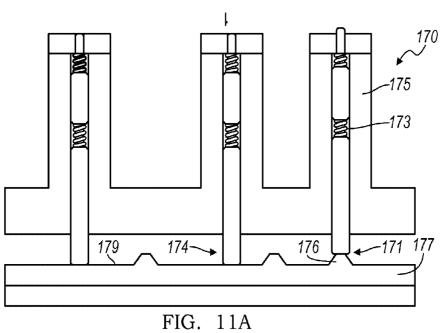
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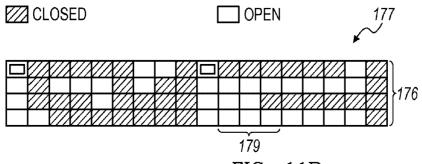
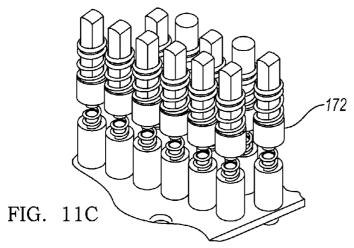


FIG. 11B



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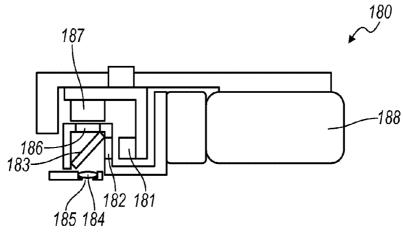


FIG. 12A

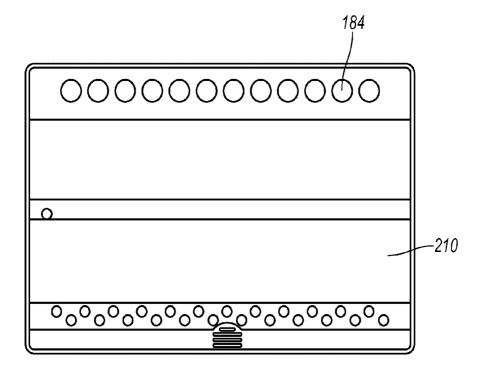
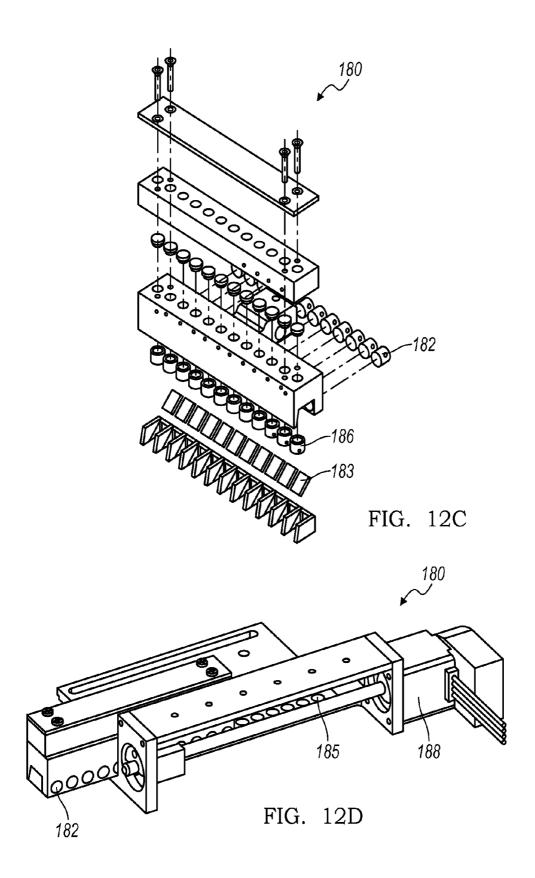


FIG. 12B

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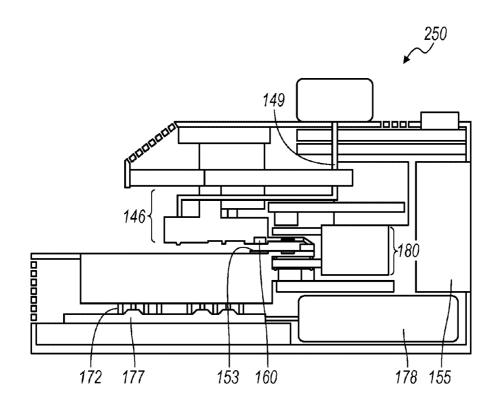
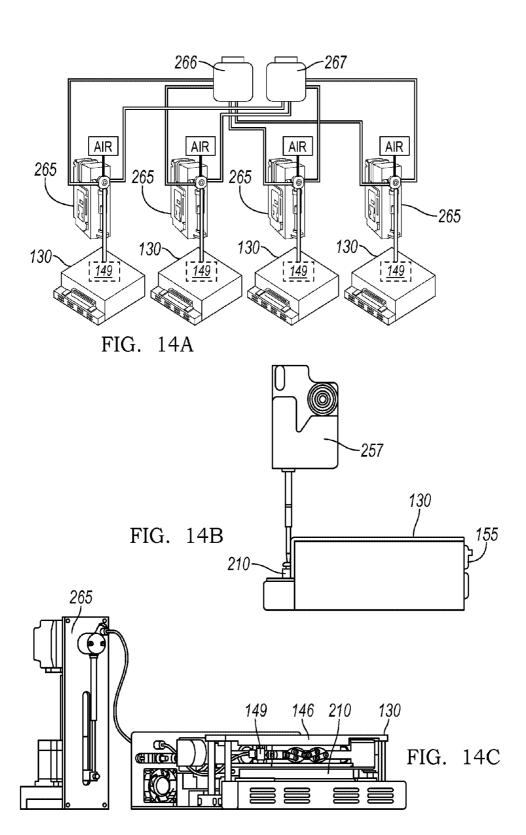


FIG. 13

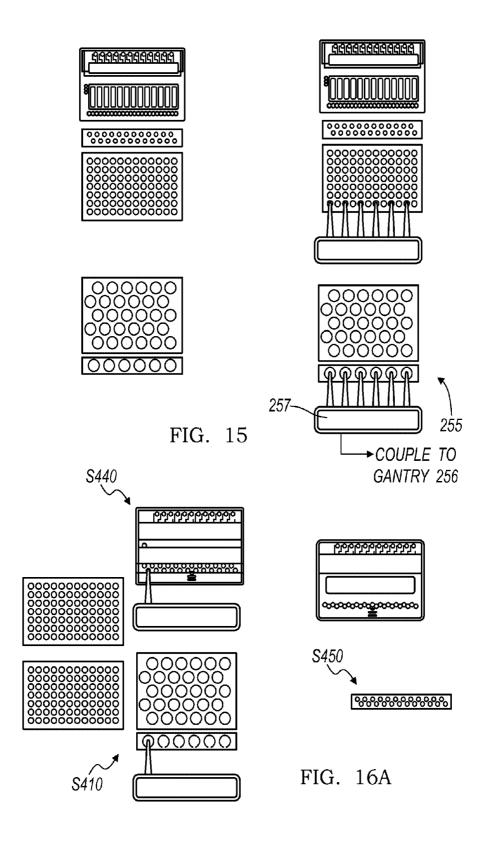
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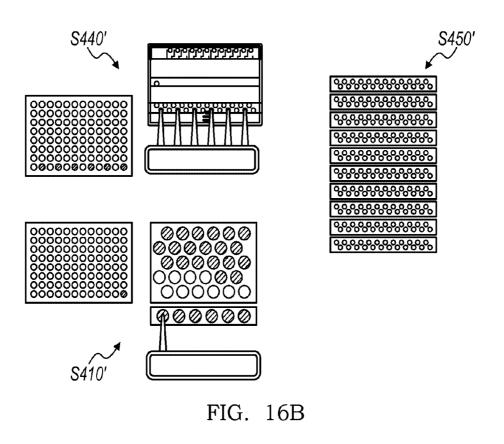
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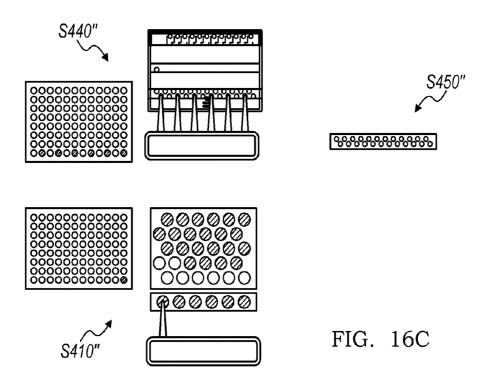


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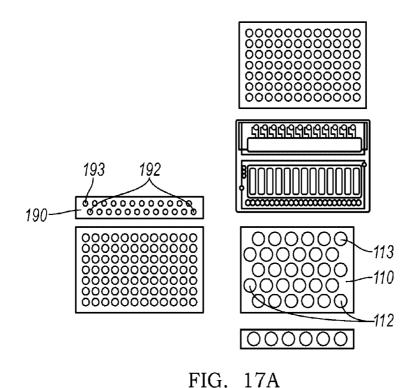
**Sheet 16 of 24** 

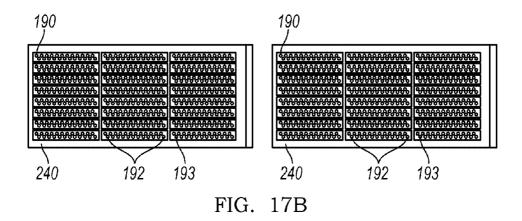




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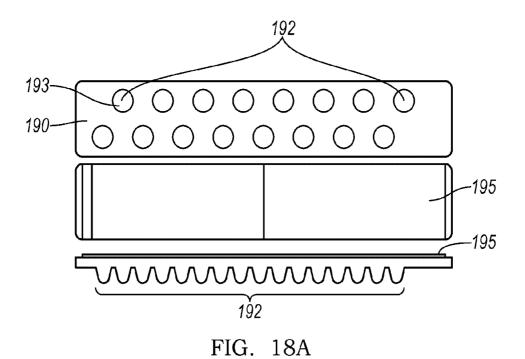




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FIG. 18B

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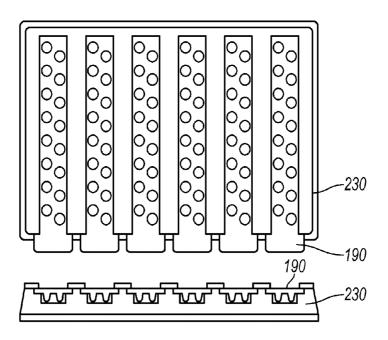
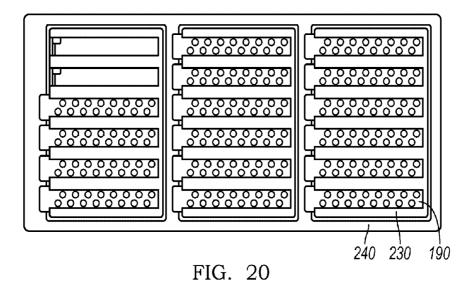


FIG. 19



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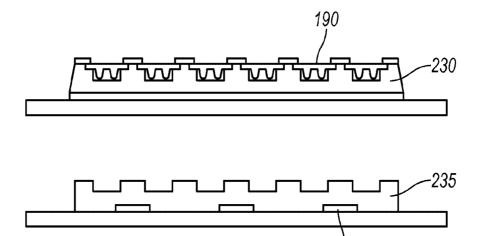


FIG. 21A

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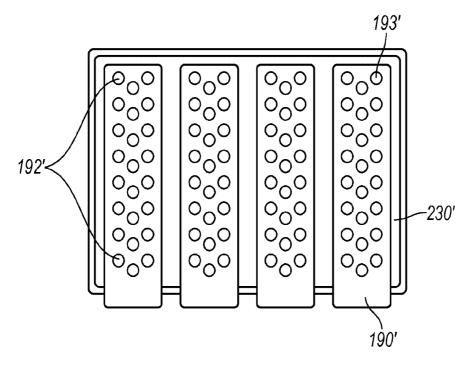
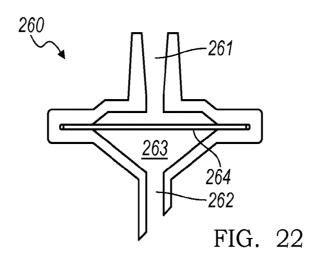
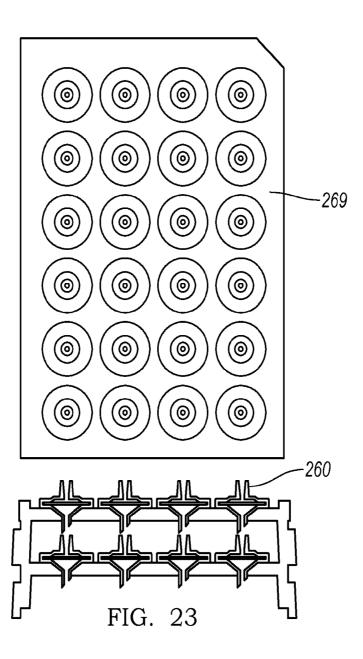


FIG. 21B

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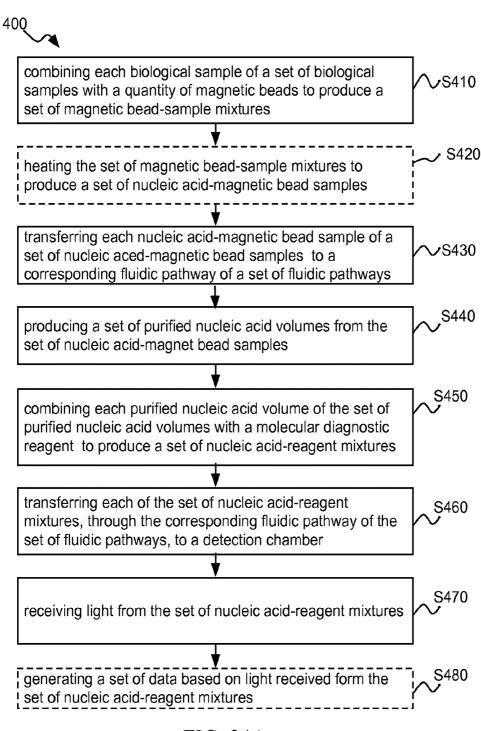
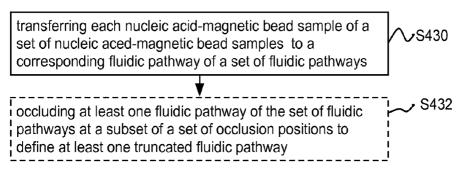
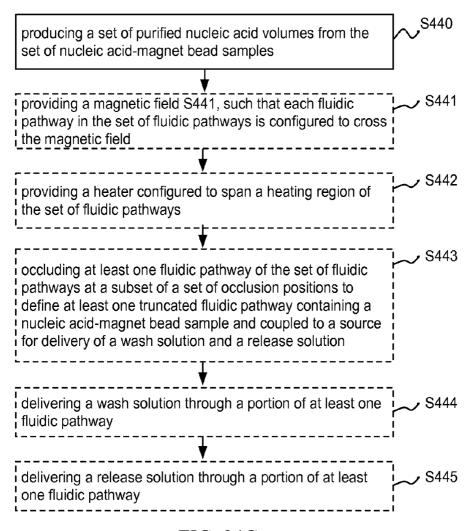


FIG. 24A

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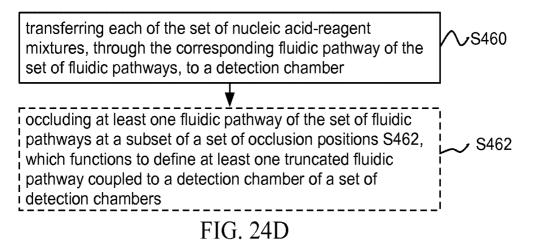
**FIG. 24B** 



**FIG. 24C** 

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#### 1

#### SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

#### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are incorporated herein in their entirety by  $\,^{10}$ this reference. This application is also related to U.S. application Ser. No. 13/765,996, which is incorporated herein in its entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved system and method for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a clinical laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology 25 research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields involving analysis of nucleic acids. Molecular diagnostic 30 analysis of biological samples can include the detection of one or more nucleic acid materials present in the specimen. The particular analysis performed may be qualitative and/or quantitative. Methods of analysis typically involve isolation, purification, and amplification of nucleic acid materials, and 35 polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/ or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecu- 40 lar diagnostic techniques are often labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are specific to certain sample matrices and/or and nucleic acid types.

Due to these and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for and improved system and method for processing and detecting nucleic acids. This invention provides such a system and 50 method.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1B depict an embodiment of a system for pro- 55 cessing and detecting nucleic acids;

FIGS. 2A-2B depict an embodiment of elements, and a top view of an embodiment of a system worktable, respectively, of an embodiment of a system for processing and detecting nucleic acids;

FIGS. 3A-3B depict an embodiment of a capture plate for combining a sample with magnetic beads;

FIG. 4 depicts an embodiment of a capture plate module to facilitate lysis of a biological sample and combination of the biological sample with magnetic beads;

FIGS. 5A-5B depict an alternative embodiment of a capture plate;

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FIGS. 6A-6B depict embodiments of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 7A-7E depict a sequence of operations performed by elements of an embodiment of a molecular diagnostic mod-

FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform;

FIGS. 9A-9B depict configurations of a linear actuator of an embodiment of a molecular diagnostic module;

FIGS. 10A-10B depict elements of an embodiment of a valve actuation subsystem of a molecular diagnostic module;

FIGS. 11A-11C depict an embodiment of a valve actuation subsystem of a molecular diagnostic module;

FIGS. 12A-12D depict elements of an embodiment of an 15 optical subsystem of a molecular diagnostic module;

FIG. 13 depicts a side view of an alternative embodiment of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 14A-14C depict an embodiment of a fluid handling 20 system of a system for processing and detecting nucleic acids; FIG. 15 depicts embodiments of elements of the fluid handling system;

FIGS. 16A-16C are schematics depicting example methods for processing and detecting nucleic acids;

FIGS. 17A-17B show embodiments of consumables and reagents used in a system for processing and detecting nucleic acids;

FIGS. 18A-18B depict an embodiment of an assay strip to facilitate analysis of a sample containing nucleic acids;

FIG. 19 depicts an embodiment of an assay strip holder; FIG. 20 depicts an embodiment of an assay strip carrier; FIGS. 21A-21B show alternative embodiments of assay strip holders and assay strips, respectively;

FIG. 22 shows an embodiment of a filter to facilitate processing and detecting of nucleic acids;

FIG. 23 shows an embodiment of a filter holder to facilitate processing and detecting of nucleic acids; and

FIGS. 24A-24D depict embodiments of a method for processing and detecting nucleic acids.

#### DESCRIPTION OF THE PREFERRED **EMBODIMENTS**

The following description of preferred embodiments of the nucleic acid types and not applicable across common sample 45 invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

1. System for Processing and Detecting Nucleic Acids

As shown in FIGS. 1A-1B and 7A, an embodiment of a system 100 for processing and detecting nucleic acids comprises: a capture plate 110 configured to facilitate binding of nucleic acids within a biological sample to a set of magnetic beads 119; a molecular diagnostic module 130 comprising a microfluidic cartridge receiving module 140, heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, an optical subsystem 180; and an assay strip 190 configured to facilitate mixing of molecular diagnostic reagents with a nucleic acid volume. Other embodiments of the system 100 may further comprise at least one of a capture 60 plate module 120 configured to support the capture plate 110; a filter 200 and filter holder 205 to facilitate sample preparation; a microfluidic cartridge 210 configured to facilitate sample processing; an assay strip holder 230; an assay strip carrier 240; a liquid handling system 250 configured to facilitate gas and fluid delivery to different elements of the system 100; a processor configured to analyze data resulting from a run of the system 100; and a user interface configured to allow

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a user to interact with the system 100. The system 100 thus functions to receive biological samples containing nucleic acids (i.e., impure nucleic acid samples), separate nucleic acids from the biological samples, and analyze nucleic acid samples according to at least one molecular diagnostic protocol (e.g., PCR). Preferably, the system 100 is a walkaway system by which a user loads a set of biological samples containing nucleic acids, and receives a set of data resulting from a molecular diagnostic protocol without any further sample manipulation by the user. Alternatively, the system 100 facilitates aspects of sample preparation for a molecular diagnostic protocol, with some sample manipulation performed by the user.

In one example workflow of the system 100, a liquid handling system 250 aspirates a set of biological samples con- 15 taining nucleic acids (i.e., impure nucleic acid samples), and dispenses the set of biological samples into a capture plate 110 to be lysed and combined with magnetic beads (containing a proprietary affinity coating to bind the nucleic acids to the magnetic beads) by a capture plate module 120. The liquid 20 handling system 250 then aspirates substantially all of each sample of the set of lysed biological samples combined with magnetic beads (i.e., set of magnetic bead-samples) from the capture plate no, and dispenses the set of magnetic beadsamples into a microfluidic cartridge 210, aligned within a 25 cartridge receiving module 140 of a molecular diagnostic module 130, and configured to be manipulated by the molecular diagnostic module 130. A heating and cooling subsystem 150, a magnet 160, and a valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate separation of 30 a set of nucleic acids from the magnetic bead-samples, as the liquid handling system 250 dispenses wash solutions, release solutions, and/or air at appropriate stages. The liquid handling system 250 then aspirates the set of nucleic acids from the microfluidic cartridge 210 contained within the molecular 35 diagnostic module 130, combines the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, and dispenses the set of nucleic acids combined with the set of molecular diagnostic reagents (i.e., set of nucleic acidreagent mixtures) into the microfluidic cartridge 210 within 40 the molecular diagnostic module 130. The detection chamber heaters 157, optical subsystem 180 and valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user 45 interface.

As stated, the above workflow is just one example workflow of the system 100, and other workflows of the system 100 and methods of processing and detecting nucleic acid samples are further described in Section 2 below. A detailed 50 description of elements of an embodiment of the system 100 are described in sections 1.1-1.6 below.

#### 1.1 System—Capture Plate and Capture Plate Module

As shown in FIGS. 3A and 3B, the capture plate 110 comprises a capture plate substrate in comprising a set of 55 wells 112 and a puncturable foil seal 115, and functions to facilitate binding of nucleic acids within a biological sample to a set of magnetic beads 119. Preferably, the entire capture plate 110 is configured to be a consumable (i.e., disposable), such that each well of the capture plate 110 can only be used once yet the remaining unused wells can be used during additional runs of the system 100. Alternatively, at least a portion of the capture plate 110 is configured to be reusable, such that additional mixing or reagent additions can be performed and portions of the capture plate 110 may be used for 65 multiple runs of the system 100. In one variation of the capture plate 110, the capture plate substrate 111 is reusable,

while the puncturable foil seal 115 is disposable and replaced after each run of the system 100.

The capture plate substrate 111 is configured such that the capture plate 110 is capable of resting on a flat surface, can be stacked with another capture plate 110, and also can be manipulated with industry standard instrument components for handling of microtiter plates. The capture plate substrate also functions to define the set of wells 112 and to couple to the puncturable foil seal 115. The capture plate substrate in is preferably composed of a PCR-compatible polymer that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 112 of the capture plate substrate 111 function to receive at least one biological sample which contain or are suspected of potentially containing nucleic acids, and to facilitate combination of the biological sample with a set of magnetic beads 119. Preferably, the wells 113 are each configured to accommodate not only a biological sample, but also to facilitate mixing of the biological sample with a set of magnetic beads 119 (e.g., using a pipettor, the liquid handling system 250 or other apparatus), which preferably are preloaded in wells 112, or alternatively may be added by an operator. Preferably, the wells are also deeper than they are wide to allow a significant number of wells 112 (e.g. 24) with a clinically relevant sample volumes, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wider than they are deep to facilitate larger devices for mixing the biological samples with the magnetic beads 119. Each well 113 of the set of wells 112 also preferably has a conically shaped bottom region, as shown in FIG. 3A, to facilitate complete aspiration of a fluid from a well. Alternatively, each well 113 may not have a conically shaped bottom region. Additionally, in the orientation shown in FIG. 3A, the tops of each well 113 in the set of wells 112 preferably form raised edges protruding from the capture plate substrate 111, in order to facilitate sealing of each well 113 by the puncturable foil seal 115. Alternatively, the tops of each well 113 in the set of wells 112 may not form raised edges protruding from the capture plate substrate in. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. However, the magnetic beads may alternatively be any appropriate magnetic beads (e.g. magnetic, parmagnetic, or superparamagnetic) configured to facilitate biomagnetic separation.

Each quantity of magnetic beads 119 may be accompanied by lysing reagents (e.g. proteinase K) and a sample process control comprising nucleic acid sequences for DNA and RNA, which function to lyse biological samples and to provide a mechanism by which sample process controls may be later detected to verify processing fidelity and assay accuracy. The sample process control comprising nucleic acid sequences for DNA and RNA allows one version of the capture plate to facilitate assays involving DNA and RNA detection. Preferably, the quantity of magnetic beads 119, lysing reagents, and sample process controls is dried within each well to improve shelf life; however, the quantity of magnetic beads 119, lysing reagents, and sample process controls may alternatively be in liquid form.

The puncturable foil seal 115 functions to isolate each well 113 of the set of wells 112, prevent contamination of the contents of each of the set of wells 112, protect the magnetic beads 119 and other reagents stored in wells 112 from deg-

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radation, and provide information identifying the capture plate no. The puncturable foil seal 115 preferably seals each well 113 of the capture plate 110, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one 5 variation, the puncturable foil seal 115 also forms a seal around an element that punctures it, and in another variation, the puncturable foil seal 115 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 115 is also preferably labeled with identifying information including at least one of manufacturer information, capture plate contents, the lot of the contents, an expiry date, and a unique electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal 115 does not extend beyond the footprint of the 15 capture plate no, but alternatively, the puncturable foil seal 115 may be any appropriate size and/or include protruding features (e.g., tabs) that facilitate handling of the capture

In one variation, the capture plate no may be prepackaged at least with magnetic beads 119, such that each well 113 in the set of wells 112 is prepackaged with a set of magnetic beads 119 defined by a specific quantity or concentration of magnetic beads. The set of wells 112 may then be sealed by the puncturable foil seal 115, which is configured to be punctured by an external element that delivers volumes of biological samples to be mixed with the magnetic beads 119. In another variation, the capture plate 110 may not be prepackaged with magnetic beads 119, but the wells 113 of the capture plate may still be sealed with a puncturable foil seal 115. In this variation, the puncturable foil seal 115 is configured to be punctured by at least one external element, for co-delivery of biological samples and magnetic beads intended to be combined.

A variation of the capture plate 110' may further comprise a slotted rubber membrane 116, as shown in FIGS. 5A and 5B, configured to provide access through the puncturable foil seal 115 to the set of wells 112. The slotted rubber membrane 116 thus functions to prevent or reduce splashing, evaporation, and/or aerosolization of contents of the set of wells 112. 40 Preferably, the slotted rubber membrane 116 comprises slots that are self-sealing and centered over wells of the set of wells 112, and further does not extend beyond the footprint of the capture plate no. Alternatively, the slots of the slotted rubber membrane 116 may not be self-sealing, and/or the slotted rubber membrane 116 may be any appropriate size and comprise features that extend beyond the footprint of the capture plate no.

In a specific example, the capture plate no comprises 24 wells 113 with an 18 mm center-to-center pitch, each well 50 having a volumetric capacity of 2 mL, and is compliant with Society for Laboratory Automation and Screening (SLAS) standards. Each well 113 of the capture plate no in the specific example is also prepackaged with a specified quantity of magnetic beads 119, and comprises a protruding top edge that 55 is heat sealed to a puncturable foil seal. In addition, each well 113 also contains other reagents beneficial for processing and monitoring the sample, including proteinase K and one or more specific nucleic acid stands designed to serve as a process control. The specific example of the capture plate 110 60 can thus combine two groups of 12 biological samples with magnetic beads. The capture plate 110 in the specific example is produced by injection molding, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier.

An embodiment of the system 100 may further comprise a capture plate module 120, as shown in FIG. 4, which func-

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tions to receive, support, and heat a capture plate 110. The capture plate module 120 preferably comprises a thermally conducting substrate 121 configured to cradle a capture plate 110, a capture plate heater 123, a capture plate receiving module 125, and a capture plate electronics module 127. Preferably, the capture plate module 120 functions to facilitate lysis of a biological sample deposited into a well 113 of the capture plate, and to facilitate binding of nucleic acids (i.e., within a lysed biological sample) to a quantity of magnetic beads 119 within a well 113 of the capture plate 110. In a specific example, the capture plate module 120 has dimensions of 108 mm×156 mm×45 mm and is configured to rest on a flat surface.

The thermally conducting substrate 121 is configured to cradle and support the capture plate 110, and functions to conduct heat to the set of wells 112 of the capture plate 110. Preferably, the thermally conducting substrate 121 is also configured to reversibly couple to the capture plate 110, and comprises a set of indentations 122 that encircle each well 113 in the set of wells 112. In one variation, the indentations 122 completely conform to the external surface of each well 113 of the capture plate 110, but in another variation, the indentations 122 may encircle a portion of each well 113 of the capture plate 110. Additionally, the indentations 122 are preferably thermally conducting in order to conduct heat to the set of wells 112, and portions of the thermally conducting substrate 121 aside from the indentations 122 are composed of non-conducting, rigid material. Alternatively, the entire thermally conducting substrate 121 may be composed of a material that is thermally conducting.

The capture plate heater 123 is preferably coupled to the thermally conducting substrate 121, and functions to transfer heat, through the thermally conducting substrate 121, to a well 113 of the capture plate 110. The capture plate heater 123 preferably conforms to at least a portion of an indentation 122 of the thermally conducting substrate 121, to facilitate heat transfer through the indentation 122 to an individual well 113 of the capture plate 110. In this variation, the capture plate heater 123 is one of a set of capture plate heaters 124, wherein each capture plate heater 123 in the set of capture plate heaters 124 transfers heat to an individual well 113 of the set of wells 112 of the capture plate 110. Alternatively, the capture plate heater 123 may conform to portions of multiple indentations 122 of the thermally conducting substrate 121, such that the capture plate heater 123 is configured to transfer heat to multiple wells 113 of the capture plate 110. Preferably, the capture plate heater 123 is a resistance heater, but alternatively, the capture plate heater 123 may be a Peltier or any appropriate heater configured to transfer heat to the capture plate 110. The capture plate heater 123 may also further couple to a heat sink.

The capture plate receiving module 125 comprises a capture plate actuation system 126 that functions to couple the capture plate module 120 to a capture plate 110. As shown in FIG. 4, the capture plate actuation system 126 comprises a structural support with hinged grips 128 and at least one capture plate module actuator 129. The capture plate module actuator 129 is preferably a push-type solenoid with a spring return, but may alternatively be any appropriate linear actuator, such as a hydraulic actuator. The structural support with hinged grips 128 preferably couples to the capture plate heater 123 and houses the capture plate module actuator 129, such that, in a first configuration, actuation of the capture plate module actuator 129 outwardly displaces the hinged grips (allowing the capture plate module 120 to receive a capture plate 110), and in a second configuration, actuation of the capture plate module actuator 129 inwardly displaces the

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hinged grips (allowing the capture plate module 120 to couple to the capture plate 110). The structural support with hinged grips 128 may further comprise a textured and/or high-friction surface configured to grip a capture plate 110, but alternatively may not comprise a textured and/or high-friction surface.

The capture plate electronics module 127 is coupled to the capture plate heater 123 and the capture plate actuation system 126, and functions to enable control of the capture plate heater 123 and the capture plate actuation system 126. Preferably, the capture plate electronics module 127 modulates an output of the capture plate heater 123, in order to controllably heat at least one well 113 of the capture plate 110. Additionally, the capture plate electronics module 127 preferably modulates the capture plate actuation system 126, in order to 15 controllably couple the capture plate module 120 to a capture plate 110. Preferably, the capture plate electronics module 127 is coupled to an external power supply, such that the capture plate module 120 does not include an integrated power supply; however, in alternative embodiments, the cap- 20 ture plate electronics module 127 may be coupled to a power supply integrated with the capture plate module 120. 1.2 System—Molecular Diagnostic Module

As shown in FIGS. 6A and 6B, an embodiment of the molecular diagnostic module 130 of the system 100 includes 25 a cartridge receiving module 140, a heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, and an optical subsystem 180, and functions to manipulate a microfluidic cartridge 210 for processing of a biological sample containing nucleic acids. The molecular diagnostic 30 module 130 is preferably configured to operate in parallel with at least one other molecular diagnostic module 130, such that multiple microfluidic cartridges 210 containing biological samples may be processed simultaneously. In a first variation, the molecular diagnostic module 130 is configured to be 35 stackable with another molecular diagnostic module 130 in a manner that enables access to a microfluidic cartridge 210 within each molecular diagnostic module 130; an example of the first variation is shown in FIG. 6B, where the molecular diagnostic modules 130 are stacked in a staggered configu- 40 ration. In the first variation, each molecular diagnostic module 130 may further comprise locking pins or other appropriate mechanisms to couple the stacked molecular diagnostic modules 130 together. In another variation, the molecular diagnostic module 130 may not be configured to stack with 45 another molecular diagnostic module, such that the molecular diagnostic modules 130 are configured to rest side-by-side on the same plane. Elements of an embodiment of the molecular diagnostic module 130 are further described in sections 1.2.1 to 1.2.5 below.

1.2.1 Molecular Diagnostic Module—Cartridge Receiving Module

As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge platform 141 including a cartridge loading guiderail 142, a 55 cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141, and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol. As shown in FIGS. 7A-7C, the cartridge platform 141 is preferably configured to receive a microfluidic cartridge 210 along a cartridge loading 65 guiderail 142 until it reaches a cartridge stop 143, and be vertically displaced by the linear actuator 146, which places a

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biasing force against the set of springs 148 coupled to the cartridge platform 141. The magnet receiving slot 144 and the set of valve actuation slots 145 provide access, by a magnet 160 and a valve actuation subsystem 170, to the microfluidic cartridge 210, as the microfluidic cartridge is vertically displaced by the linear actuator 146.

The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment. Alternatively, the cartridge loading guiderails 142 and the cartridge stop 143 may be configured such that a microfluidic cartridge slides over or along the cartridge loading guiderails 142, after which the cartridge stop 143 couples to a portion of the microfluidic cartridge 210 to ensure proper alignment of the microfluidic cartridge. Additional variations of the cartridge loading guiderails 142 and the cartridge stop 143 may be used to enable reception and alignment of a microfluidic cartridge 210 by the molecular diagnostic module 130, and are known by those skilled in the alt.

The embodiment of the cartridge platform 141 shown in FIG. 8 also includes a set of valve actuation slots 145, oriented perpendicular to the parallel cartridge loading guiderails 142 and configured to provide access to a valve actuation subsystem 170, and a magnet receiving slot 144 located among the set of valve actuation slots 145. Preferably, the magnet receiving slot 144 and the set of valve actuation slots 145 substantially span a long dimension of the cartridge platform 141, as shown in FIG. 8, and are configured to correspond to locations on a microfluidic cartridge 210 requiring a magnetic field and/or valving to enable processing of a biological sample and nucleic acid detection once the microfluidic cartridge 210 has been aligned within the molecular diagnostic module 130. Thus, alternative configurations of the magnet 50 receiving slot 144 and the set of valve actuation slots 145 may accommodate other cartridges with alternative regions requiring magnetic fields and/or valving to enable other protocols. In one alternative embodiment, the magnet receiving slot 144 and the set of valve actuation slots may comprise one continuous void of the cartridge platform 141, such that the cartridge platform 141 supports a microfluidic cartridge 210 along the periphery of the microfluidic cartridge 210, but forms a continuous void under a majority of the footprint of the microfluidic cartridge 210.

The linear actuator 146 functions to linearly displace a microfluidic cartridge 210 resting on the cartridge platform 141, in order to compress the microfluidic cartridge 210 and position the microfluidic cartridge 210 between a cartridge heater 153 and an optical subsystem 180 on one side of the microfluidic cartridge 210, and a magnet 160 and detection chamber heaters 157 on another side of the microfluidic cartridge 210. The linear actuator 146 also functions to provide

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a sufficient counterforce to the valve actuation subsystem 170 such that a microfluidic cartridge 210 within the molecular diagnostic module 130 remains properly situation upon manipulation by the valve actuation subsystem 170. The linear actuator 146 further functions to move a nozzle 149 5 coupled to the liquid handling system 250, in order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210. In the orientation of the molecular diagnostic module 130 shown in FIGS. 7B and 7B, the linear actuator **146** is preferably coupled to a portion of the heating and cooling subsystem 150 a portion of the optical subsystem 180, and the nozzle 149, and vertically displaces the cartridge heater 153, the optical subsystem 180, and the nozzle 149 to position the cartridge heater 153, 180 and the nozzle 149 over the microfluidic cartridge 210. The vertical displacement also 15 allows the microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the 20 nucleic acid (e.g. PCR). Preferably, the linear actuator 146 is a scissor jack actuator configured to apply substantially uniform pressure over all occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and to operate in at least two configurations. In a 25 retracted configuration 146a, as shown in FIG. 9A, the scissor jack actuator has not linearly displaced the cartridge platform 141, and in an extended configuration 146b, as shown in FIG. 9B, the scissor jack actuator has linearly displaced the microfluidic cartridge 210 to position the microfluidic cartridge 210 30 between the subsystems 153, and 180, and the magnet 160 and detection chamber heaters 157. Additionally, the extended configuration 146b of the scissor jack actuator is configured to couple the nozzle 149 to a fluid port 222 of the microfluidic cartridge 210, such that the liquid handling sys- 35 tem 250 can deliver solutions and gases for processing of biological samples. The linear actuator 146 may alternatively be any appropriate linear actuator, such as a hydraulic, pneumatic, or motor-driven linear actuator, configured to linearly displace a microfluidic cartridge within the molecular diag- 40 nostic module 130.

As shown in FIGS. 7B, 7C, and 8, a set of springs 148 is coupled to the cartridge platform 141 and functions to provide a counteracting force against the linear actuator 146 as the linear actuator 146 displaces a microfluidic cartridge 210 45 resting on the cartridge platform 141. The set of springs 148 thus allows the cartridge platform 141 to return to a position that allows the microfluidic cartridge 210 to be loaded and unloaded from the molecular diagnostic module 130 when the linear actuator 146 is in a retracted configuration 146b, as 50 shown in FIG. 7B. Preferably, in the orientation shown in FIG. 7B, the set of springs 148 is located at peripheral regions of the bottom side of the cartridge platform 141, such that the set of springs 148 does not interfere with the magnet or the valve actuation subsystem 170. Alternatively, the set of 55 springs 148 may be located at any appropriate position to provide a counteracting force against the linear actuator 146. In a specific example shown in FIG. 6A, the set of springs 148 comprises four springs located near corners of the bottom side of the cartridge platform 141, but in other variations, the 60 set of springs 148 may comprise any appropriate number of springs. Each spring of the set of springs 148 is also preferably housed within a guide to prevent deviations from linear vertical motions (in the orientation shown in FIG. 7B); however, each spring in the set of springs 148 may alternatively 65 not be housed within a guide. In an alternative embodiment of the molecular diagnostic module 130, the set of springs 148

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may altogether be replaced by a second linear actuator configured to linearly displace a microfluidic cartridge 210, resting on the cartridge platform 141, in a direction opposite to the displacements enforced by the linear actuator 146.

Similarly, the nozzle 149, the heating and cooling subsystem 150, the cartridge heater 153, and the magnet 160 are preferably coupled to springs, such that springs are positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. Alternatively an elastomeric material is preferably positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. The springs and/or elastomeric material function to provide proper functioning and alignment of subsystems of the molecular diagnostic module 130 as the linear actuator 146 is extended or retracted, contributing to reliability and a reduction in stack up tolerance risk. The springs and/or elastomeric material further function to allow more pressure to be applied to occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and an appropriate pressure to be applied to elements 149, 150, 153 and 160 of the molecular diagnostic module 130. Thus, proper contact is maintained between elements 149, 150, 153, and 160, and a microfluidic cartridge 210 being manipulated by the molecular diagnostic module. These elements are described in further detail below.

1.2.2 Molecular Diagnostic Module—Heating/Cooling Subsystem and Magnet

The heating and cooling subsystem 150 of the molecular diagnostic module 130 comprises a cartridge heater 153, a fan 155, and a set of detection chamber heaters 157 and functions to controllably heat portions of a microfluidic cartridge 210 for processing of a biological sample containing nucleic acids according to a molecular diagnostic protocol. In the orientation of an embodiment of the molecular diagnostic module 130 shown in FIGS. 7A-7C, the cartridge heater 153 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140 and configured to span a central region of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, the fan 155 is located at a back wall of the cartridge receiving module 140, and the set of detection chamber heaters 157 is located inferior to a set of detection chambers 213 of the microfluidic cartridge 210. In alternative embodiments of the molecular diagnostic module 130, the heating and cooling subsystem 150 may have any appropriate alternative configuration that provides controlled heating and cooling to a microfluidic cartridge within the molecular diagnostic module 130.

The cartridge heater 153 functions to transfer heat to a heating region 224 of a microfluidic cartridge 210, for inducing a pH shift to release bound nucleic acids from magnetic beads within the heating region 224. The cartridge heater 153 is preferably a plate-shaped heater configured to transfer heat to the microfluidic cartridge 210 only from one side of the cartridge heater 153, such that heat flows through one face of the plate-shaped heater to the microfluidic cartridge 210. In a specific example, the cartridge heater 153 is a silicon wafer etched to be conductive and form a resistance heater. In the preferred variation, the cartridge heater 153 is either flip-chip bonded (i.e., soldered to back side of a circuit board), or wire bonded to a circuit board, and then coupled using linear bearings and springs to a plate coupled to the linear actuator 146. The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing. In another variation, heating through one face is accomplished using a plate-shaped resistance heater that has one exposed face and thermal insulation

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covering all other faces, and in yet another variation heating through one face is accomplished using a Peltier heater. In a variation of the cartridge heater 153 using a Peltier heater, the cartridge heater 153 comprises a thermoelectric material, and produces different temperatures on opposite faces of the cartridge heater 153 in response to a voltage difference placed across the thermoelectric material. Thus, when a current flows through the Peltier heater, one face of the Peltier heater lowers in temperature, and another face of the Peltier heater increases in temperature. Alternative variations of the cartridge heater 153 can be used to appropriately transfer heat to a heating region 224 of the microfluidic cartridge 210.

Preferably, the cartridge heater 153 is configured to linearly translate with the linear actuator 146 of the cartridge receiving module 140, in order to align with a heating region 15 224 spanning a central portion of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In one variation, the cartridge heater 153 is preferably fixed relative to the linear actuator 146 such that (in the orientation shown in FIGS. 7B-7C), the cartridge heater 153 can only move 20 vertically with the linear actuator. In an alternative variation, the cartridge heater 153 may additionally be configured to translate laterally with a horizontal plane (in the orientation shown in FIGS. 7B-7C), such that the cartridge heater 153 can translate in at least two perpendicular coordinate planes. In 25 this alternative variation, the cartridge heater 153 can be configured to sweep across a surface of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, or to translate in response to motion of the microfluidic cartridge 210, such that the position of the cartridge heater 30 153 relative to a heating region 224 of the microfluidic cartridge 210 is always fixed.

The fan 155 functions to modulate heat control within the molecular diagnostic module 130, by enabling heat transfer from warm objects within the molecular diagnostic module 35 130 to cooler air external to the molecular diagnostic module 130. In the orientation shown in FIG. 6A, the fan 155 is preferably located at a back face of the molecular diagnostic module 130, such heat within the molecular diagnostic module 130 is transferred out of the back face of the molecular 40 diagnostic module 130 to cooler air external to the molecular diagnostic module. In a specific embodiment, the molecular diagnostic module 130 comprises four fans 155 located at the back face of the molecular diagnostic module 130; however, in alternative embodiments the molecular diagnostic module 45 130 may comprise any appropriate number of fans located at any appropriate position of the molecular diagnostic module 130. In one variation, the fan 155 may be passive and driven solely by convection currents resulting from motion of hot air within the molecular diagnostic module to cooler air outside 50 of the molecular diagnostic module; however, in alternative variations, the fan 155 may be motor-driven and configured to actively cool internal components of the molecular diagnostic module 130 if molecular diagnostic module elements exceed a certain threshold temperature.

The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210. Each detection chamber heater in the set of detection chamber heaters 157 is preferably configured to heat one side of one 60 detection chamber in the set of detection chambers 213, and is preferably located such that the extended configuration 146b of the linear actuator 146 of the cartridge receiving module 140 puts a detection chamber in proximity to a detection chamber heater. As mentioned above, the set of detection 65 chamber heaters 157 is preferably coupled to springs or an elastomeric layer to ensure direct contact between the set of

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detection chamber heaters and a set of detection chambers, without compressively damaging the set of detection chamber heater 157. Preferably, each detection chamber heater is configured to contact a surface of a detection chamber in the extended configuration 146b of the linear actuator 146; however, each detection chamber heater may be further configured to couple to a detection chamber in the extended configuration 146b of the linear actuator 146. In a first variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a flexible printed circuit board, with a set of springs coupled to an opposite surface of the flexible printed circuit board, such that each spring in the set of springs aligns with a detection chamber heater. In the first variation, contact between each detection chamber heater and a detection chamber is thus maintained by a biasing force provided by an individual spring through the flexible printed circuit board. In a second variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a rigid printed circuit board, with a set of springs coupled to an opposite surface of the rigid printed circuit board. In the second variation, the set of springs thus function to collectively transfer a force through the rigid printed circuit board to maintain contact between the set of detection chamber heaters and a set of detection chambers. Preferably, each detection chamber heater in the set of detection chamber heaters 157 is configured to contact and heat a bottom surface of a detection chamber (in the orientation shown in FIG. 7B); however, each detection chamber heater may alternatively be configured to contact and heat both a top and a bottom surface of a detection chamber. Additionally, each detection chamber heater preferably corresponds to a specific detection chamber of the set of detection chambers 213 and functions to individually heat the specific detection chamber; however, alternatively, each detection chamber heater may be configured to heat multiple detection chambers in the set of detection chambers 213. Preferably, all detection chamber heaters in the set of detection chamber heaters 157 are identical; however, the set of detection chamber heaters 157 may alternatively not comprise identical detection chamber heaters.

In one variation, each detection chamber heater in the set of detection chamber heaters 157 comprises a donut-shaped heater, configured to encircle a surface of a detection chamber. The donut-shaped heater may further include a conducting mesh configured to allow detection through the heater while still allowing efficient heat transfer to the detection chamber. In an alternative variation, each detection chamber heater in the set of detection chamber heaters 157 may include a plate-shaped Peltier heater, similar to Peltier cartridge heater 153 described above. In this alternative variation, each detection chamber heater is thus configured to heat one side of a detection chamber through one face of the detection chamber heater. In one specific example, the molecular diagnostic module 130 comprises 12 diced silicon wafers with conductive channels flip chipped to 12 detection chambers, providing resistive heating to each of the 12 detection chambers. In another specific example, the molecular diagnostic module 130 comprises a 12 Peltier detection chamber heaters configured to heat 12 detection chambers of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In other alternative variations, each detection chamber heater may comprise any appropriate heater configured to individually heat a detection chamber.

The magnet 160 of the molecular diagnostic module 130 functions to provide a magnetic field for isolation and extraction of nucleic acids bound to magnetic beads within a microfluidic cartridge 210, aligned within the molecular

diagnostic module 130. Preferably, the magnet 160 is fixed within the molecular diagnostic module 130, such that the extended configuration 146b of the linear actuator 146 allows the magnet 160 to pass through the magnet receiving slot 144 of the cartridge receiving module 140 and into a magnet 5 housing region 218 of the microfluidic cartridge 210. In an example, as shown in FIGS. 7A-7C, the magnet 160 is a rectangular prism-shaped magnet 160 fixed under the cartridge platform 141, and configured to pass through the cartridge platform 141, into a magnet housing region 218 located under the heating region 224 of the microfluidic cartridge 210. Preferably, the magnet 160 is one of two or three magnets lined up in parallel, such that each of the fluidic pathways of a microfluidic cartridge housing the magnets is exposed to two or three times as much magnetic flux, and two to threes 15 times as many opportunities to capture magnetic beads. Alternatively, the magnet 160 is a single magnet configured to expose a set of fluidic pathways to a magnetic field. Preferably, the magnet 160 or group of multiple magnets is coupled

130. Additionally, the magnet holder is preferably composed

of an insulating material, such that the magnet holder does not

interfere with proper functioning of the cartridge heater 153.

Alternatively, the magnet holder may not be composed of an

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insulating material. In one variation, the magnet 160 or group of multiple magnets comprises a permanent magnet, composed of a magnetized material (e.g., a ferromagnet) providing a substantially fixed magnetic field. In an alternative variation, the magnet 160 or group of multiple magnets comprises an elec- 30 tromagnet configured to provide a modifiable magnetic field, such that the intensity of the magnetic field can be adjusted, the polarity of the magnetic field can be reversed, and the magnetic field can be substantially removed upon removal of a current flowing within the electromagnet. Preferably, the 35 magnet 160 or group of magnets is also fixed relative to the molecular diagnostic module 130; however, the magnet 160 or group of magnets may alternatively be configured to translate vertically (in the orientation shown in FIG. 7B), such that the magnet 160 or group of magnets can extend into and 40 retract from the magnet receiving slot 144 of the cartridge platform 141 and the magnet housing region 218 of the microfluidic cartridge 210. Additionally, the magnet 160 or group of magnets preferably rides on linear bearings and springs (or an elastomeric material) to ensure proper contact 45 with a microfluidic cartridge in an extended configuration 146b of the linear actuator 146, in a manner that allows most of force from the linear actuator 146 to translate to full occlusion of a subset of the set of occlusion positions (i.e., without leakage)

Alternative configurations and/or compositions of the magnet 160 may also be appropriate in facilitating isolation and extraction of nucleic acids bound to magnetic beads within the microfluidic cartridge 210.

1.2.3 Molecular Diagnostic Module—Valve Actuation Sub- 55 system

As shown in FIGS. 10A-11C, the valve actuation subsystem 170 of the molecular diagnostic module 130 comprises a set of pins 172 configured to translate linearly within a pin housing 175, by sliding a cam card 177 laterally over the 60 pins 172. The valve actuation subsystem 170 functions to provide a biasing force to deform an object in contact with the set of pins 172. In a configuration wherein a microfluidic cartridge 210 is aligned within the molecular diagnostic module 130, the valve actuation subsystem 170 thus functions to 65 occlude a fluidic pathway 220 of the microfluidic cartridge 210 at a set of occlusion positions 226, to control flow of a

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biological sample containing nucleic acids, reagents and/or air through the microfluidic cartridge 210. In an embodiment of the molecular diagnostic module shown in FIGS. 7D-7E, the set of pins 172 and the pin housing are located directly under the microfluidic cartridge 210, such that the set of pins can access the microfluidic cartridge 210 through the valve actuation accommodating slots 145 of the cartridge platform 141. The cam card 177 in the embodiment is positioned under the set of pins and is coupled to a linear cam card actuator 178 configured to laterally displace the cam card 177 to vertically displace pins of the set of pins 172. Preferably, as shown in FIG. 11A, the cam card 177 rests on a low friction surface configured to facilitate lateral displacement of the cam card 177; however, the cam card 177 may alternatively rest on a bed of ball bearings to facilitate lateral displacement of the cam card 177, or may rest on any feature that allows the cam card 177 to be laterally displaced by the linear cam card actuator 178

The cam card 177, as shown in FIGS. 7D and 11A, includes to a magnet holder within the molecular diagnostic module 20 a set of hills 176 and valleys 179, and functions to transform linear motion in one plane to vertical motion in another plane. In one variation, the cam card 177 is coupled to a linear actuator and contacts the ends of pins in a set of pins 172, such that when a hill 176 of the cam card 177 passes under a pin, the pin is in a raised configuration 177a, and when a valley 179 of the cam card 177 passes under a pin, the pin is in a lowered configuration 177b. The hills 176 and valleys 179 of the cam card 177 are preferably in a set configuration, as shown in FIG. 11B, such that lateral motion of the cam card 177 to a set position raises a fixed subset of the set of pins 172. In this manner, lateral movement of the cam card 177 to different positions of a set of positions consistently raises different subsets of the set of pins 172 to occlude different portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172. Thus, portions of a fluidic pathway 220 may be selectively occluded and opened to facilitate processing of a biological sample according to any appropriate tissue, cellular, or molecular diagnostic assay protocol. In one variation, the cam card is configured to be laterally displaced in two coordinate directions within a plane (e.g., by x-y linear actuators), and in another variation, the cam card is configured to be laterally displaced in only one coordinate direction within a plane (e.g., by a single linear actuator). In a specific example, the hills 176 of the cam card 177 are raised 1 mm above the valleys 179 of the cam card 177, the hills 176 and valleys 179 each have a 2 mm wide plateau region, and a hill 176 region slopes down to a valley region 179 at a fixed angle over a 2 mm length. In the specific example, the cam card 177 is driven by a Firgelli linear actuator. Alternative variations may include any appropriate configurations and geometries of a cam card with hills 176 and valleys 179, driven by any appropriate actuator.

In alternative embodiments of the valve actuation subsystem 170, the cam card 177 may be a cam card wheel comprising a set of hills 176 and valleys 179 on a cylindrical surface, and configured to convert rotary motion to linear (i.e., vertical) motion of the set of pins 172. The cam card wheel may be configured to contact ends of pins in the set of pins 172, and may be coupled to a motor shaft and driven by a motor. In other alternative embodiments of the valve actuation subsystem 170, the cam card 177 may altogether be replaced by a set of cams, each configured to individually rotate about an axis. In these alternative embodiments, rotating subsets of the set of cams raises corresponding subsets of the set of pins, and occludes specific portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172.

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The set of pins 172 functions to selectively occlude portions of a fluidic pathway 220 of a microfluidic cartridge 210 at least at subsets of a set of occlusion positions 226. The pins of the set of pins 172 are preferably cylindrical and, in the orientation shown in FIG. 11A, configured to slide over a cam 5 card 177 and within a pin housing 175. Each pin in the set of pins 172 preferably also includes a first spring 173 that functions to provide a counteracting force to restore a pin to a lowered configuration 177b; however, each pin in the set of pins 172 may alternative not include a first spring 173, and 10 rely solely on gravity to return to a lowered configuration 177b. Preferably, as shown in FIG. 11C, each pin is also composed of two parts separated by a second spring, which functions to allow sufficient force to fully occlude a microfluidic channel but prevents forces from being generated that 15 could damage the pin, microfluidic cartridge and/or cam card. Each pin also preferably comprises a first region 171 configured to slide within the pin housing 175, and a second region 174 configured to exit the pin housing 175. The second region 174 is preferably of a smaller dimension than the first region 20 171, such that each pin is constrained by the pin housing 175 to be raised by a limited amount. Alternatively, the first region 171 and the second region 174 may have any appropriate configuration to facilitate raising and lowering of a pin by a fixed amount. In a specific example, the valve actuation sub- 25 system 170 comprises 12 sets of pins 172 configured to selectively occlude 12 fluidic pathways 212 of a microfluidic cartridge 210 aligned within the molecular diagnostic module; however, other embodiments may comprise any appropriate number of sets of pins 172.

In the orientation shown in FIG. 11A, each pin in the set of pins 172 preferably has a circular cross section and round ends, configured to facilitate sliding within a pin housing 175, sliding over a cam card 177 surface, and occlusion of a fluidic pathway 220. Alternatively, each pin may comprise any 35 appropriate cross-sectional geometry (e.g., rectangular) and/ or end shape (e.g., flat or pointed) to facilitate occlusion of a fluidic pathway 220. Preferably, the surface of each pin in the set of pins 172 is composed of a low-friction material to facilitate sliding motions (i.e., over a cam card 177 or within 40 a pin housing 175); however, each pin may alternatively be coated with a lubricant configured to facilitate sliding motions.

The pin housing 175 functions to constrain and guide the motion of each pin in the set of pins 172, as the cam card 177 slides under the set of pins 172. Preferably, the pin housing 175 comprises a set of pin housing channels 169 configured to surround at least one pin in the set of pins 172. In one variation, each pin in the set of pins 172 is surrounded by an individual channel of the set of pin housing channels 169; 50 however, in another variation a channel of the set of pin housing channels 169 may be configured to surround multiple pins in the set of pins 172. In an example shown in FIGS. 7D-7E and 11A, the pin housing is located under the cartridge platform 141, such that the set of pin housing channels 169 is 55 aligned with the set of valve actuation accommodating slots 145, to provide access, by the set of pins 172, to a microfluidic cartridge 210 aligned on the cartridge platform 141. In the example, the pin housing 175 thus constrains the set of pins 172, such that each pin can only move linearly in a vertical 60 direction. Each pin housing channel preferably has a constricted region 168 configured to limit the motion of a pin within a pin channel; however, each pin housing channel may alternatively not include a constricted region. Preferably, surfaces of the pin housing 175 contacting the set of pins 172 are 65 composed of a low friction material to facilitate sliding of a pin within a pin housing channel; however, surfaces of the pin

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housing 175 contacting the set of pins 172 may alternatively be coated with a lubricant configured to facilitate sliding motions. Other variations of the pin housing 175 and the set of pins 172 may include no additional provisions to facilitate sliding of a pin within a pin housing channel.

1.2.4 Molecular Diagnostic Module—Optical Subsystem

As shown in FIGS. 12A-12D, the optical subsystem 180 of the molecular diagnostic module 130 comprises a set of light emitting diodes (LEDs) 181, a set of excitation filters 182 configured to transmit light from the set of LEDs 181, a set of dichroic mirrors 183 configured to reflect light from the set of excitation filters 182 toward a set of apertures 185 configured to transmit light toward a set of nucleic acid samples, a set of emission filters 186 configured to receive and transmit light emitted by the set of nucleic acid samples, and a set of photodetectors 187 configured to facilitate analysis of light received through the set of emission filters 186. The optical subsystem 180 may further comprise a set of lenses 184 configured to focus light onto the set of nucleic acid samples. The optical subsystem 180 thus functions to transmit light at excitation wavelengths toward a set of nucleic acid samples and to receive light at emission wavelengths from a set of nucleic acid samples. Preferably, the optical subsystem 180 is coupled to an optical subsystem actuator 188 configured to laterally displace and align the optical subsystem 180 relative to the set of nucleic acid samples, and is further coupled to a linear actuator 146 of the cartridge receiving module 140 to position the optical subsystem 180 closer to the set of nucleic acid samples. Alternatively, the optical subsystem 180 may not be coupled to a linear actuator 146 of the cartridge receiving module 140, and may only be configured to translate laterally in one direction. In a specific example, the optical subsystem 180 comprises a set of 12 apertures, a set of 12 lenses, a set of 12 dichroic mirrors, a set of 12 excitation filters, a set of 12 LEDs, a set of 12 emission filters, and a set of 12 photodetectors. In the specific example, as shown in FIGS. 7A-7E, the optical subsystem 180 is located within the molecular diagnostic module 130 and coupled to the linear actuator 146 of the cartridge receiving module 140, such that, in the extended configuration 146b of the linear actuator 146, the optical subsystem 180 can be positioned closer to a microfluidic cartridge 210 aligned within the molecular diagnostic module. Conversely in the specific example, the optical subsystem 180 is positioned away from the microfluidic cartridge 210 in the retracted configuration 146a of the linear actuator 146. In the specific example, the optical subsystem 180 is further coupled to an optical subsystem actuator 188 configured to laterally displace the optical subsystem 180 relative to the microfluidic cartridge 210, such that the optical subsystem 180 can be aligned with a set of detection chambers 213 of the microfluidic cartridge 210.

Preferably, the set of LEDs 181 are not all identical but rather chosen to efficiently produce a certain band of wavelengths of light, such that light from the set of LEDs  $181\ \mathrm{can}$ be filtered to appropriate narrow wavelengths for analysis of nucleic acid samples. Alternatively, all LEDs in the set of LEDs 181 may be identical, and produce white light comprising all wavelengths of visible light that is filtered to produce the desired wavelength, in which case the LEDs may be stationary. Preferably, the set of LEDs 181 includes phosphor-based LEDs, but the set of LEDs 181 may alternatively include any LEDs configured to provide light of the desired range of wavelengths. The LEDs of the set of LEDs 181 are preferably configured to emit light of wavelengths corresponding to at least one of the set of excitation filters 182, the set of dichroic mirrors 183, and the set of emission filters 186.

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The set of excitation filters 182 is configured to align with the set of LEDs 181 in the optical subsystem 180, and functions to transmit light at excitation wavelengths toward the set of dichroic mirrors 183 of the optical subsystem 180. Preferably, the set of excitation filters 182 are not identical excitation filters, but rather chosen to transmit the different desired ranges of excitation wavelengths. Alternatively, all excitation filters of the set of excitation filters 182 are identical, and configured to transmit light having a fixed range of excitation wavelengths. In one variation, the set of excitation filters 182 includes band pass filters, configured to transmit light between two bounding wavelengths, in another variation, the set of excitation filters 182 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of excitation filters 182 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of excitation filters 182 is interchangeable, such that individual excitation filters may be interchanged to provide different excitation wavelengths of light; however, the set of excitation filters 182 may alterna- 20 tively be fixed, such that the optical subsystem 180 is only configured to transmit a fixed range of excitation wavelengths.

The set of dichroic mirrors 183 is configured to align with the set of excitation filters 182, and functions to receive and 25 reflect light from the set of excitation filters 182 toward the detection chamber, such that light having a range of excitation wavelengths may be focused, through a set of apertures, onto a set of nucleic acid samples. The set of dichroic mirrors 183 also functions to receive and transmit light from a set of 30 emission filters 186 toward a set of photodetectors 187, which is described in more detail below. All dichroic mirrors in the set of dichroic mirrors 183 are preferably identical in orientation relative to the set of excitation filters 182 and the set of emission filters 186, and configured to reflect and transmit the 35 appropriate wavelengths of light for the given LED. Alternatively, the set of dichroic mirrors 183 may include identical dichroic mirrors, with regard to orientation, light transmission, and light reflection. In a specific example, in the orientation shown in FIG. 12A, the set of excitation filters 182 is 40 oriented perpendicular to the set of emission filters 186, with the set of dichroic mirrors 183 bisecting an angle between two planes formed by the faces of the set of excitation filters 182 and the set of emission filters 186. In the specific example, light from the set of excitation filters is thus substantially 45 reflected at a 90° angle toward the set of apertures 185, and light from the set of emission filters 186 passes in a substantially straight direction through the set of dichroic mirrors 183 toward the set of photodetectors 187. Other variations of the set of dichroic mirrors 183 may include any configuration of 50 dichroic mirrors, excitation fillers, and/or emission filters that enable transmission of light of excitation wavelengths toward a set of nucleic acid samples, and transmission of light from the set of nucleic acid samples toward a set of photodetectors

In one embodiment, the optical subsystem may further include a set of lenses 184 configured to align with the set of dichroic mirrors 183, which functions to focus light, from the set of excitation filters 182 and reflected off of the set of figured to emit light in response to the light from the set of excitation filters 182. All lenses in the set of lenses 184 are preferably identical in orientation relative to the set of dichroic mirrors and in dimension; however, the set of lenses 184 may alternatively comprise non-identical lenses, such that 65 light passing through different lenses of the set of lenses 184 is focused differently on different nucleic acid samples. In a

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specific example, in the orientation shown in FIG. 12A, the faces of the set of lenses 184 are oriented perpendicular to the faces of the set of excitation filters 182, to account for light reflection by the set of dichroic mirrors 183 at a 90° angle. In the specific example, the set of lenses also includes identical 1/4" high numerical aperture lenses. In other variations, the set of lenses 184 may be oriented in any appropriate configuration for focusing light from the set of dichroic mirrors 183 onto a set of nucleic acid samples, and may include lenses of any appropriate specification (i.e., numerical aperture).

The set of apertures 185 is located on an aperture substrate 189 and configured to align with the set of lenses 184, and functions to allow focused light from the set of lenses 184 to pass through to the set of nucleic acid samples. The aperture substrate 189 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140, which allows the optical subsystem 180 to linearly translate and be positioned near and away from a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. Alternatively, the aperture substrate 189 may not be coupled to the linear actuator 146 of the cartridge receiving module 140. Preferably, all apertures 185 in the set of apertures 185 are identical, and configured to allow identical light profiles to be focused, through the set of lenses 184, onto a set of nucleic acid samples. Alternatively, the set of apertures 185 may not include identical apertures. In one variation, each aperture in the set of apertures 185 may be individually adjustable, in order to provide individually modifiable aperture dimensions (e.g., width, length, or diameter) to affect light exposure. In an alternative variation, each aperture in the set of apertures 185 is fixed. Other variations may include interchangeable aperture substrates 189, such that features of the set of apertures (e.g., aperture dimensions, number of apertures) may be adjusted by interchanging aperture substrates 189.

The set of emission filters 186 is configured to align with the set of dichroic mirrors, and functions to transmit emission wavelengths of light from the set of nucleic acid samples, and to filter out excitation wavelengths of light. Preferably, each emission filter of the set of emission filters 186 are configured to transmit light having a fixed range of emission wavelengths, while blocking light of excitation wavelengths. Alternatively, the set of emission filters 186 may comprise identical emission filters, such that individual emission filters of the set of emission filters 186 are configured to transmit the same ranges of emission wavelengths. In one variation, the set of emission filters 186 includes band pass fillers, configured to transmit light between two bounding wavelengths, in another variation, the set of emission filters 186 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of emission filters 186 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of emission filters 186 is interchangeable, such that individual emission filters may be interchanged to transmit and/or block different wavelengths of light; however, the set of emission filters 186 may alternatively be fixed, such that the optical subsystem 180 is only configured to transmit a fixed range of emission wavelengths.

The set of photodetectors 187 is configured to align with dichroic mirrors 183, onto a set of nucleic acid samples con- 60 the set of emission filters 186, and functions to receive light from the set emission filters to facilitate analysis of the set of nucleic acid samples. All photodetectors in the set of photodetectors 187 are preferably identical; however, the set of photodetectors 187 may alternatively include non-identical photodetectors. Preferably, the set of photodetectors 187 includes photodiodes comprising a photoelectric material configured to convert electromagnetic energy into electrical

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signals; however, the set of photodetectors 187 may alternatively comprise any appropriate photodetectors for facilitating analysis of biological samples, as is known by those skilled in the art.

The optical subsystem actuator 188 is coupled to the opti- 5 cal subsystem 180, and functions to laterally translate the optical subsystem 180 relative to a set of nucleic acid samples being analyzed. Preferably, the optical subsystem actuator 188 is a linear actuator configured to translate the optical subsystem 180 in one dimension; however, the optical subsystem actuator 188 may alternatively be an actuator configured to translate the optical subsystem 180 in more than one dimension. In a specific example, as shown in FIGS. 7A-7D and 12D, the optical subsystem actuator 188 is configured to translate the optical subsystem 180 laterally in a horizontal 15 plane, to align the optical subsystem 180 with a set of detection chambers 213 of a microfluidic cartridge 210 within the molecular diagnostic module 130. In another example, the optical subsystem may be configured as a disc revolving around an axis with the LEDs and photodetectors stationary 20 and the disc containing the filters. In other variations, the optical subsystem actuator 188 may be configured in any appropriate manner to facilitate alignment of the optical subsystem 180 relative to a set of nucleic acid samples being analyzed.

1.2.5 Molecular Diagnostic Module—Alternative Embodiments and Variations

As described above, alternative embodiments of the molecular diagnostic module 130 and alternative variations of subsystems and elements of the molecular diagnostic module 130 may be configured to process a biological sample containing nucleic acids, isolate nucleic acids from the biological sample, and detect nucleic acids. An example of an alternative embodiment of a molecular diagnostic module 130, as shown in FIG. 13, includes a cartridge receiving 35 module 140', a heating and cooling subsystem 150', a magnet 160', a valve actuation subsystem 170', and an optical subsystem 180', and functions to manipulate an alternative microfluidic cartridge 210' for processing of biological samples containing nucleic acids. Other alternative embodi- 40 ments of the molecular diagnostic module 130" may be configured to receive alternative microfluidic cartridges 210", for processing of biological samples containing nucleic acids. 1.3 System—Assay Strip

As shown in FIGS. 18A and 18B, the assay strip 190 45 comprises an assay strip substrate 191 comprising a set of wells 192, and typically a puncturable foil seal 195, and functions to facilitate combination of a set of nucleic acid samples with a set of molecular diagnostic reagents for amplification and/or detection of a nucleic acid sequence or 50 sequences. Preferably, the entire assay strip 190 is configured to be a consumable (i.e., disposable), such that the assay strip 190 can be used during multiple runs of the system 100, then the assay strip 190 is disposed of once all of the wells 192, containing unitized reagents for a single test or group of tests, 55 is exhausted. Alternatively, at least a portion of the assay strip 190 is configured to be reusable, such that wells may be reloaded with reagents and reused with the system 100. In one variation of the assay strip 190, the assay strip substrate 191 is reusable, while the puncturable foil seal 195 is disposable and 60 replaced after each run of the system 100. In another variation, the reusable assay strip substrate 191 does not require a puncturable foil seal 195, such that reagents specific to a certain nucleic acid sequences may be deposited into open wells of the assay strip substrate 191 by a user.

The assay strip substrate 191 is configured such that the assay strip 190 is capable of resting on a flat surface, and

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functions to define the set of wells 192 and to couple to the puncturable foil seal 195. The assay strip substrate 191 is preferably configured to be received by a corresponding assay strip holder 230 configured to hold multiple assay strips 190, but may alternatively not be configured to couple to an assay strip holder 230. The assay strip substrate 191 is preferably composed of a PCR-compatible polymer, such as polypropylene, that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 192 of the assay strip substrate 191 function to receive at least one nucleic acid sample, and to facilitate combination of the nucleic acid sample with at least one of a set of molecular diagnostic reagents. The molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to perform alternative molecular diagnostic protocols. Preferably, the wells 193 of the assay strip substrate 191 are each configured to accommodate not only a nucleic acid sample, but also to facilitate mixing of the nucleic acid sample with at least one of a set of molecular diagnostic reagents (e.g., using a pipettor or other apparatus). Additionally, the molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprises probes and primers to detect the sample process controls provided by the capture plate, in order to verify process fidelity and assay accuracy. Preferably, the wells 193 are deep enough to facilitate mixing without splashing, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wide and shallow to facilitate drying of reagents in the wells to increase shelf life and larger devices for mixing the nucleic acids with molecular diagnostic reagents. Each well 193 of the set of wells 192 also preferably has a rounded bottom region, as shown in FIG. 18A, to facilitate complete aspiration of a fluid from a well 193; however, each well 193 may alternatively not have a rounded bottom region. Additionally, the set of wells 192 is preferably arranged in staggered rows, which functions to facilitate access to individual wells 193 of the set of wells, to reduce one dimension of the assay strip 190, and also to prevent cross-contamination of fluids within the wells due to dripping. Alternatively, the set of wells 192 may not be arranged in staggered rows.

The puncturable foil seal 195 functions to protect the molecular diagnostic reagents stored in wells 112 from degradation, isolate each well 193 of the set of wells 192, prevent contamination of the contents of each of the set of wells 192, and provide information identifying the assay strip 190. The puncturable foil seal 195 preferably seals each well 193 of the assay strip 190, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one variation, the puncturable foil seal 195 also forms a seal around an element that punctures it, and in another variation, the puncturable foil seal 195 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 195 is also preferably labeled with identifying information including at least one of manufacturer information, assay strip contents, the lot of the contents, an expiry date, and a unique

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electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal **195** does not extend beyond the footprint of the assay strip **190**, but alternatively, the puncturable foil seal **195** may be any appropriate size and/or include protruding features (e.g., tabs) that <sup>5</sup> facilitate handling of the assay strip.

In one variation, the assay strip 190 may be prepackaged with a set of molecular diagnostic reagents, such that each well 193 in the set of wells 192 is prepackaged with a quantity of molecular diagnostic reagents. The set of wells 192 may then be sealed by the puncturable foil seal 195, which is configured to be punctured by an external element that delivers volumes of nucleic acid samples to be combined with the set of molecular diagnostic reagents. In another variation, the assay strip 190 may not be prepackaged with a set of molecular diagnostic reagents, and the wells 193 of the assay strip 190 may not be sealed with a puncturable foil seal 195. In yet another variation, the system may comprise an empty assay strip 190 without a puncturable foil seal 195, and an assay 20 strip 190 comprising reagents and a puncturable foil seal 195, such that a user may add specific reagents to the empty assay strip to be used in conjunction with the assay strip comprising reagents. In variations comprising a puncturable foil seal 195, the puncturable foil seal 115 is configured to be punctured by 25 at least one external element, for co-delivery of nucleic acid samples and molecular diagnostic reagents intended to be combined.

In a specific example, the assay strip 190 has an 87 mm×16 mm footprint and comprises 24 wells 113 arranged in two 30 staggered rows, with a 9 mm center-to-center pitch between adjacent wells 193 within each row. Each well 193 of the set of wells has a capacity of 60 µL to accommodate a volume of a molecular diagnostic reagent, 20 µL of a sample fluid, and any displacement caused by a pipette tip (e.g., 100 or 300 μL 35 pipette tip). Each well 113 of the assay strip 190 in the specific example is also prepackaged with a quantity of molecular diagnostic reagents, and comprises a protruding top edge (75 microns high) that is heat sealed to a puncturable foil seal. The capture plate no in the specific example is produced by injec- 40 tion molding, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier. In the specific embodiment, the vapor barrier is further increased by depositing a thin metallic layer to the outside of the assay strip 190.

As described earlier, the assay strip 190 may be configured to be received by an assay strip holder 230. The assay strip holder 230 functions to receive and align multiple assay strips 190, such that a multichannel pipettor or other fluid delivery system may combine multiple nucleic acid samples with 50 molecular diagnostic reagents using wells 193 of multiple assay strips 190. In one variation, the assay strip holder 230 may be configured to contain Assay strips 190 including reagents for substantially different molecular diagnostic assays, as shown in FIG. 17B, such that a single run of the 55 system 100 involves analyzing a set of nucleic acid samples under different molecular diagnostic assays. In another variation, the assay strip holder 230 may be configured to contain assay strips 190 including reagents for identical molecular diagnostic assays, such that a single run of the system 100 involves analyzing a set of nucleic acid samples under the same molecular diagnostic assay. Preferably, the assay strip holder 230 is composed of a material that is dishwasher safe and autoclavable, configured to hold the assay strips 190 in place during handling by a fluid delivery system (e.g., pipettor), and configured such that the assay strips 190 avoid protruding over an edge of the assay strip holder 230, but the

assay strip holder 230 is constructed to facilitate insertion and removal of the assay strips 190 from the assay strip holder

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In one variation, the assay strip holder 230 is not configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190; however, in another variation as shown in FIG. 21A, the assay strip holder 230 may be further configured to couple to an aluminum block 235 coupled to a set of Peltier units 236 configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190. Additionally, the assay strip holder 230 may be configured to be received and carried by an assay strip carrier 240, which, as shown in FIG. 20, functions to facilitate handling and alignment of multiple assay strip holders 230. In a specific example, as shown in FIG. 19, the assay strip holder 230 has dimensions of 127.76 mm×85.48 mm×14.35 mm, complies with American National Standards Institute (ANSI) and Society for Laboratory Automation and Screening (SLAS) standards, and is configured to hold six 16-well assay strips for a total of 96 wells **193**. In another specific example, as shown in FIG. 21B, the assay strip holder 230' is configured to hold four assay strips 190', each comprising 24 wells 193' for a total of 96 wells per assay strip holder 230'. Other combinations of the described embodiments, variations, and examples of the assay strip 190, assay strip holder 230, and assay strip carrier 240 may be incorporated into embodiments of the system 100 for processing and detecting nucleic acids. 1.4 System—Microfluidic Cartridge

magnetic bead-samples, facilitate separation of nucleic acids from the set of magnetic bead-samples, receive a set of nucleic acid-reagent samples, and facilitate analysis of nucleic acids from the set of nucleic acid-reagent samples. In one embodiment, the microfluidic cartridge 210 comprises a top layer 211 including a set of sample port-reagent port pairs 212 and a set of detection chambers 213; an intermediate substrate 214, coupled to the top layer 211 and partially separated from the top layer 211 by a film layer 215, configured to form a waste chamber 216; an elastomeric layer 217 partially situated on the intermediate substrate 214; a magnet housing region 218 accessible by a magnet 160 providing a magnetic field; and a set of fluidic pathways 219, each formed by at least a portion of the top layer 211, a portion of the film layer 215, and a portion of the elastomeric layer 217. In the embodiment, the microfluidic cartridge 10 further comprises a bottom layer 221 coupled to the intermediate substrate 214 and configured to seal the waste chamber 216. Furthermore, in the embodiment, the top layer 211 of the microfluidic cartridge 210 further comprises a shared fluid port 222, a vent region 223, and a heating region 224, such that each fluidic pathway 220 in the set of fluidic pathways 219 is fluidically coupled to a sample port-reagent port pair 224, the shared fluid port 222, the waste chamber 216, and a detection chamber 225, comprises a turnabout portion 226 configured to pass through the heating region 224 and the magnetic field, and is configured to pass through the vent region 223 upstream of

The microfluidic cartridge 210 functions to receive a set of

The microfluidic cartridge 210 is preferably configured to be received and manipulated by the molecular diagnostic module 130, such that the cartridge receiving module 140 of the molecular diagnostic module 130 receives and aligns the microfluidic cartridge 210 within the molecular diagnostic module 130, the heating and cooling subsystem 150 of the molecular diagnostic module 130 is configured to transfer

the detection chamber 225. Each fluidic pathway 220 thus

functions to receive and facilitate processing of a sample fluid

containing nucleic acids as it passes through different por-

tions of the fluidic pathway 220.

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heat to the heating region 224 of the microfluidic cartridge 210, and the magnet 160 of the molecular diagnostic module 130 is configured to be received by the magnet housing region 218 of the microfluidic cartridge 210 to provide a magnetic field for separation of nucleic acids. Additionally, the shared fluid port 222 of the microfluidic cartridge 210 is configured to couple to a nozzle 149 coupled to the linear actuator 146 of the cartridge receiving module 140, such that the liquid handling system 250 can deliver fluids and gases through the shared fluid port 222. The elastomeric layer 217 of the microfluidic cartridge 210 is also preferably configured to be occluded at a set of occlusion positions 226 by the valve actuation subsystem 170 of the molecular diagnostic module, in order to occlude portions of a fluidic pathway 220 of the microfluidic cartridge 210 for processing of a set of biological samples. The optical subsystem 180 of the molecular diagnostic module 130 is further configured to align with the set of detection chambers 213 of the microfluidic cartridge 210, to facilitate analysis of a set of nucleic acid samples. The microf-20 luidic cartridge 210 is preferably the microfluidic cartridge 210 described in U.S. application Ser. No. 13/765,996, which is incorporated in its entirety by this reference, but may alternatively be any appropriate cartridge or substrate configured to receive and process a set of samples containing nucleic 25

#### 1.5 System—Fluid Handling System and Filter

The liquid handling system 250 of the system 100 includes a liquid handling arm 255 and a syringe pump 265, as shown in FIGS. 14A-14C and functions to deliver biological 30 samples, reagents, and gases to elements of the system 100. As described in Section 1, an embodiment of the liquid handling system 250 is configured to aspirate a set of biological samples containing nucleic acids (i.e., impure nucleic acid samples), dispense the set of biological samples into a capture 35 plate no to be lysed and combined with magnetic beads by a capture plate module 120, aspirate the set of biological samples combined with magnetic beads (i.e., set of magnetic bead-samples) from the capture plate 110, and dispense the set of magnetic bead-samples into microfluidic cartridge 210 40 located in a molecular diagnostic module 130. The embodiment of the liquid handling system 100 is further configured to facilitate separation of a set of nucleic acids from the magnetic bead-samples, by dispensing a wash solution, a release solution, and/or air into the molecular diagnostic 45 module 130, by the nozzle 149 coupled to the linear actuator 146, at appropriate stages, aspirate the set of nucleic acids from the molecular diagnostic module 130, combine the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, and dispense the set of nucleic acids 50 combined with the set of molecular diagnostic reagents (i.e., set of nucleic acid-reagent mixtures) into the molecular diagnostic module 130 for further processing and analysis. [Other embodiments of the liquid handling system 250 may be configured to perform alternative molecular diagnostic assay pro- 55 tocols and/or dispense and aspirate alternative fluids into and from other elements supporting a molecular diagnostic protocol.

The liquid handling arm 255 comprises a gantry 256 and a multichannel liquid handling head 257, and functions to 60 travel to different elements of the system 100 for fluid delivery and aspiration. The liquid handling arm 255 is preferably automated and configured to move, aspirate, and deliver fluids automatically, but may alternatively be a semi-automated liquid handling arm 255 configured to perform at least one of 65 moving, aspirating, and delivering automatically, while another entity, such as a user, performs the other functions.

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The gantry 256 is coupled to the multichannel liquid handling head 257, and functions to transport the multichannel liquid handling head 257 to different elements of the system 100 for fluid delivery and aspiration. Preferably, the gantry 256 is automated and configured to translate the multichannel liquid handling head 257 within at least two dimensions, and provides X-Y positional accuracy of at least 0.5 mm. Additionally, in the orientation shown in FIG. 14B, the gantry is preferably situated above the molecular diagnostic module 130, such that the gantry 256 can translate within at least two dimensions without interfering with other elements of the system 100. Alternatively, the gantry 256 may be any appropriate gantry 256 to facilitate movement of an end effector within at least two dimensions, as is readily known by those skilled in the art.

The multichannel liquid handling head 257 functions to aspirate fluids from and deliver fluids to different elements of the system 100. Preferably, the multichannel liquid handling head 257 is a multichannel pipette head; however, the multichannel liquid handling head 257 may alternatively be any appropriate multichannel liquid handling head configured to deliver fluids and/or gases. Preferably, the multichannel liquid handling head 257 comprises at least eight independent channels 258, but may alternatively comprise any number of channels 258 configured to aspirate and deliver fluids. The channel-to-channel pitch is preferably variable, and in a specific example ranges between 9 mm and 36 mm; however, the channel-to-channel pitch may alternatively be fixed, as shown in FIG. 15. The multichannel liquid handling head 257 also preferably provides independent z-axis control (in the orientation shown in FIG. 14B), such that, in combination with the gantry 256. The multichannel liquid handling head 257 is preferably configured to couple to both large (e.g., 1 mL) and small (e.g., between 100 and 300 µL) pipette tips, and in a specific example, has a precision of at least 6% using small disposable pipette tips and a precision of at least 2% using large disposable pipette tips when dispensing essentially the entire tip volume. Alternatively, the multichannel liquid handling head 257 may be configured to couple to any object configured to facilitate aspiration and delivery of fluids. Preferably, the multichannel liquid handling head 257 provides independent control of the channels 258, with regard to volumes of fluid aspirated or delivered, fluid dispensing rates, and/or engaging and disengaging pipette tips. Alternatively, the multichannel liquid handling head 257 may not provide independent control of the channels 258, such that all channels 258 of the multichannel liquid handling head 257 are configured to perform identical functions simultaneously. Preferably, the multichannel liquid handling head 257 is configured to aspirate and deliver both liquids and gases, but alternatively, the multichannel liquid handling head 257 may be configured to only aspirate and deliver liquids. Preferably, the multichannel liquid handling head 257 provides at least one of liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258; however, the multichannel liquid handling head 257 may alternatively not provide liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258.

In one embodiment, the multichannel liquid handling head 257 is configured to couple to at least one filter 260, which functions to pre-filter liquids being aspirated and/or dispensed by the liquid handling arm 255, and is preferably a custom filter 260 configured to couple to a pipette tip, but may alternatively be any appropriate filter configured to couple to the liquid handling arm 255 and filter liquids being aspirated and/or dispensed by the liquid handling arm 255.

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An embodiment of a custom filter 260, as shown in FIG. 22, comprises a first end 261 configured to couple to a pipette tip, a pointed second end 262, a void 263 coupled to the first end 261 and the pointed second end 262, and a filter membrane 264 subdividing the void 263. The first end 261, as shown in FIG. 22, preferably comprises a tapered channel configured to provide a friction fit with a pipette tip; however, the first end may alternatively not comprise a tapered channel and may be configured to couple to a pipette tip using any appropriate means. The pointed second end 262 is preferably sharp and configured to pierce an object, such as a foil seal; additionally, the pointed second end 262 is preferably at least as long as required to dispense into a well 113 of the capture plate 110. The void 263 preferably defines a conical region defined by 15 the filter membrane 264, wherein the conical region is configured to divert a fluid within the filter 260 toward the pointed second end 262; however, the void 263 may not include a conical region. The filter membrane 264 functions to filter a fluid aspirated by the multichannel liquid handling head 257, 20 and is configured to subdivide the void 263 to define a conical region; however, the filter membrane 264 may alternatively not define a conical region of the void 263. In one embodiment, in the orientation shown in FIG. 22, the region of the void **263** below the filter membrane **264** may have a volumet- 25 ric capacity of between 200 ul and 1 mL; however, the region of the void 263 below the filter membrane may alternatively have any appropriate volumetric capacity.

A set of filters **260** may further be configured to be received and delivered by a filter holder **269**, as, shown in FIG. **23**. A specific embodiment of a filter holder **269** comprises a set of 24 tapered holes with an 18 mm center-to-center pitch, arranged in six rows of four holes. The specific embodiment of the filter holder **269** is also compliant with ANSI and SLAS standards, has dimensions of 127.75×85.5×14.35 mm, and is stackable with other specific embodiments of the custom filter holder **269**. Alternatively, the filter holder **269** may be any appropriate filter holder **269** configured to receive and deliver a set of filters **260**, as is readily known by those skilled in the art.

#### 1.5.1 Fluid Handling System—Syringe Pump

The syringe pump 265 of the liquid handling system 250 is coupled to a wash solution source 266, a release solution source **267**, a source of air **268**, and flexible tubing **291**, and 45 functions to deliver a wash solution, a release solution, and air through a valve to the molecular diagnostic module 130 to facilitate isolation and purification of nucleic acids from a set of magnetic bead-samples. The flexible tubing 291 is preferably coupled at a first end to the syringe pump, and at a second 50 end to a nozzle 149 coupled to the linear actuator 146 of the molecular diagnostic module 130, as shown in FIG. 14C. As stated earlier, an extended configuration 146b of the linear actuator 146 is configured to couple the nozzle 149 to a fluid port 222 of a microfluidic cartridge 210 within the molecular 55 diagnostic module 130, such that the wash solution, release solution, and air can be delivered to the microfluidic cartridge 210 at appropriate stages. A specific embodiment of the syringe pump 265 comprises a 4-way valve, is able to pump 20-5000 μL of fluids or air through the 4-way valve at flow 60 rates from 50-500 µL/min, can couple to syringes with between 1 mL and 10 mL capacities, and has a precision of at least 5% with regard to fluid or air delivery. Alternatively, the syringe pump 265 may be any appropriate syringe pump 265 or fluid delivery apparatus configured to deliver a wash solution, a release solution, and air to the molecular diagnostic module 130, as is readily known by those skilled in the art.

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1.6 System—Additional Elements

The system 100 may further comprise a tag reader 271, which functions to read barcodes, QR codes and/or any other identifying tags of the system 100. Preferably, the tag reader 271 is coupled to the liquid handling system 250, such that the tag reader 271 is configured to read tags on puncturable foil seals 115, 195 or tags located on any element of the system 100 accessible by the liquid handling system 250; however, the tag reader 271 may alternatively not be coupled to the liquid handling system 250. In one alternative embodiment of the system 100, the tag reader 271 may be a standalone unit that is configured to be manipulated by a user to scan tags or labels located on elements of the system 100.

The system 100 may also further comprise a controller 272 coupled to at least one of the capture plate module 120, the molecular diagnostic module 130, the liquid handling system 250, and the tag reader 271, and functions to facilitate automation of the system 100. In a variation wherein the controller 272 is coupled to the capture plate module 120, the controller 272 preferably functions to automate heating of a capture plate 110, which facilitates lysing of biological samples within the capture plate 110 and binding of nucleic acids within the capture plate 110 to magnetic beads 119 of the capture plate 110. In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of nucleic acid-reagent mixtures by the optical subsystem 180. In a variation wherein the controller 272 is coupled to the liquid handling system 250, the controller 272 preferably functions to automate aspiration, transfer, and delivery of fluids and/or gases to different elements of the system 100. In a variation wherein the controller 272 is coupled to the tag reader 271, the controller preferably functions to automate reading of tags by the tag reader 271, and may further function to facilitate transfer of information from the tags to a processor 273. Other variations of a controller may function automate handling, transfer, and/or storage of other elements of the system 100, such as capture plates 110, assay strips 190, assay strip holders 230, assay strip carriers 240, filters 200, filter holders 205, and/or microfluidic cartridges 210, using a robotic arm or gantry similar to that used in the liquid handling system 250. Alternative combinations of the above variations may involve a single controller 272, or multiple controllers configured to perform all or a subset of the functions described above.

The system 100 may also further comprise a processor 273, which functions to receive and process information from a tag reader 271, and also to receive and process data received from the optical subsystem 180 of the molecular diagnostic module 130. Preferably, the processor 273 is coupled to a user interface 274, which functions to display processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information. Alternatively, the processor 273 is not coupled to a user interface 274, but comprises a connection 275 configured to facilitate transfer of processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information to a device external to the system 100.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims,

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modifications and changes can be made the described embodiments of the system 100 without departing from the scope of the system 100.

2. Method for Processing and Detecting Nucleic Acids

An embodiment of a method 400 for processing and detect- 5ing nucleic acids from a set of biological samples comprises: combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of magnetic bead-sample mixtures S410; heating the set of magnetic bead-sample mixtures to produce a set of nucleic acidmagnetic bead samples S420; transferring each nucleic acidmagnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways S430; producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples 15 S440; combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures S450; transferring each of the set of nucleic acid-reagent mixtures, through the correspond- 20 ing fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers S460; and receiving light from the set of nucleic acid-reagent mixtures S470. The method 400 may further comprise generating a set of data based on light received form the set of nucleic acid-reagent 25 mixtures S480. The method 400 functions to isolate and extract a set of nucleic acid volumes from a biological sample, and to facilitate analysis of the nucleic acid volumes according to at least one molecular diagnostic protocol.

Step S410 recites combining each biological sample of the 30 set of biological samples with a quantity of magnetic beads to produce a set of magnetic bead-sample mixtures, and functions to prepare a set of biological samples to be lysed and combined with magnetic beads. For each biological sample, Step S410 preferably comprises aspirating a portion of the 35 volume of the biological sample from a sample container (possibly containing an aqueous solution prior to addition of biological sample), and transferring the portion of the biological sample to a well containing a set of magnetic beads. Alternatively, for each biological sample, Step S410 may 40 comprise aspirating the entire volume of the biological sample from a sample container, and transferring the volume of the biological sample to be combined with a set of magnetic beads. Preferably, all biological samples in the set of biological samples are aspirated and combined with the mag- 45 netic beads in the wells simultaneously using a multichannel fluid delivery system; however, all biological samples in the set of biological samples may alternatively be aspirated and combined with a set of magnetic beads non-simultaneously. The magnetic beads are preferably polymer beads, pre- 50 coupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. However, the magnetic beads may alternatively be any appropriate magnetic beads configured to facilitate biomagnetic 55 separation.

In addition to combination with magnetic beads, Step 410 may further include combining each biological sample of the set of biological samples with a lysing enzyme (e.g. proteinase K), and a sample process control comprising two or more 60 nucleic acid sequences (i.e., one for DNA and one for RNA) to be included with each sample. This allows biological samples to effectively lysed, which releases waste components into a wash solution, and allows nucleic acids to bind to magnetic beads. This additionally allows the sample process 65 control to be later detected, as a check to verify the accuracy of a molecular diagnostic assay being performed.

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In a first variation of Step S410 for one biological sample, as shown in FIG. 16A, a volume of the biological sample is aspirated and combined with a set of magnetic beads. In the first variation of Step S410, a set of different biological samples may thus be aspirated simultaneously, and each biological sample may be transferred to an individual well to be combined with a set of magnetic beads to produce a set of magnetic bead-sample mixtures. In the first variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic bead-sample mixtures are substantially non-identical in composition. In a second variation of Step S410, as shown in FIG. 16B, a volume of a stock biological sample is aspirated, and portions of the volume of the stock biological sample are transferred to multiple wells to be combined with multiple sets of magnetic beads to produce a set of magnetic beadsample mixtures. In the second variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic beadsample mixtures are substantially identical in composition. Other variations of Step S410 may comprise filtering at least one biological sample of the set of biological samples S415 prior to combining each biological sample of the set of biological samples with a quantity of magnetic beads.

In a specific example of Step S410, a multichannel liquid handling system aspirates approximately 1 mL of each of a set of biological samples in aqueous buffer using a set of 1 mL pipette tips, couples each of the pipette tips to a custom 13 mm diameter filter, punctures a foil seal 115 of a capture plate at a set of wells, wherein each well of the set of wells contains a set of magnetic beads, and dispenses each aspirated volume of a biological sample into a well of the capture plate containing a set of magnetic beads, and disposes of the tip/filter combination. In the specific example of Step S410, the multichannel liquid handling system then picks up new disposable tips and aspirates and dispenses the contents of each well at least three times to mix the contents, and then disposes of the set of pipette tips and filters.

Step S420 recites heating the set of magnetic bead-sample mixtures to produce a set of nucleic acid-magnetic bead samples, and functions to incubate the set of magnetic beadsample mixtures in order to lyse biological matter, and release nucleic acids to be bound to magnetic beads. Preferably, Step S420 comprises heating a capture plate containing the set of magnetic bead-sample mixtures for a specified amount of time at a specified temperature, and may additionally include cooling the set of magnetic bead-sample mixtures. In a specific example, Step S420 comprises heating a capture plate containing the set of magnetic bead-sample mixtures using a capture plate module, wherein the capture plate module is configured to cradle and controllably heat wells containing the set of magnetic bead-sample mixtures. Step S420 may alternatively comprise incubating the set of magnetic beadsample mixtures using any appropriate method and/or system as is known by those skilled in the art. Finally, Step S420 may be omitted in embodiments of the method 400 involving samples that do not require heating.

Step S430 recites transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways, and functions to isolate each of the set of nucleic acid-magnetic bead samples within separate pathways for further processing. Preferably, all nucleic acid-magnetic bead samples in the set of nucleic acid-magnetic bead samples are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-magnetic bead sample in the set of magnetic bead-samples may be transferred to a corresponding fluidic pathway independently of the other nucleic acid-magnetic bead samples. In addition, preferably the

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entire volume, or substantially all of the volume, of the nucleic acid-magnetic bead sample is transferred to the set of fluidic pathways, without magnetically isolating magnetic beads and removing supernatant fluids prior to transferring each nucleic acid-magnetic bead sample of the set of nucleic 5 acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways.

Step S430 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S432, which functions to define at least one truncated fluidic pathway. Preferably, Step S432 comprises defining at least one truncated fluidic pathway passing through at least one of a heating region and a magnetic field; however, Step S432 may alternatively not comprise defining a truncated fluidic pathway passing through at least one of a heating region and a magnetic field.

In a specific example of Step S430, the multichannel liquid handling subsystem of Step S410 transfers a set of nucleic acid-magnetic bead samples to a set of fluidic pathways of a microfluidic cartridge aligned within a molecular diagnostic 20 module, wherein the microfluidic cartridge comprises an elastomeric layer in contact with the set of fluidic pathways. Manipulation of the elastomeric layer at a subset of a set of occlusion positions by a valve actuation subsystem of the molecular diagnostic module defines a set of truncated fluidic 25 pathways crossing a heating region and a magnetic field, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a truncated fluidic pathway of the set of truncated fluidic pathways.

Step S440 recites producing a set of nucleic acid volumes 30 from the set of nucleic acid-magnetic bead samples, and functions to separate nucleic acid volumes from the set of nucleic acid-magnetic bead samples. Step S440 preferably reduces a concentration of unwanted matter from the set of biological samples being processed, to an acceptable level; 35 however, Step S440 may alternatively entirely remove substantially all unwanted substances from the set of biological samples being processed. Step S440 preferably includes providing a magnetic field S441, such that each fluidic pathway in the set of fluidic pathways is configured to cross the mag- 40 netic field. Preferably, the set of nucleic acid-magnetic bead samples is captured and isolated within portions of the set of fluidic pathways crossing the magnetic field. Step S440 may further comprise providing a heater configured to span a heating region of the set of fluidic pathways S442, but may 45 alternatively comprise providing multiple heaters or altogether omit providing a heater. In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample. Step S442 functions to provide 50 a heater, which, in combination with a release solution that provides a a pH shift, facilitate a rapid and efficient unbinding of the nucleic acids from magnetic beads.

Step S440 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a 55 set of occlusion positions S443 (and opening a previously occluded channel), which functions to define at least one truncated fluidic pathway containing a nucleic acid-magnet bead sample and coupled to a source for delivery of a wash solution and a release solution. Preferably, Step S443 comprises defining at least one truncated fluidic pathway coupled to a waste chamber and to a fluid port, which functions to facilitate washing of at least one nucleic acid-magnetic bead sample, and releasing of at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. Step S440 may additionally comprise delivering a wash solution through a por-

tion of at least one fluidic pathway S444, such as the truncated fluidic pathway defined in Step S443, and delivering a release solution through a portion of at least one fluidic pathway S445, such as the truncated fluidic pathway defined in Step S443. Step S444 functions to wash at least one nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic

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magnetic bead sample in the set of nucleic acid-magnetic bead samples, and Step S445 functions to release at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. The heater provided in Step S442 may be activated after Step S445 to induce a pH shift.

In a specific example of Step S440, the set of fluidic pathways containing a set of nucleic acid-magnetic bead samples, from the specific example of Step S430, is occluded at a subset of the set of occlusion positions by a valve actuation subsystem of the molecular diagnostic module, to define a set of truncated fluidic pathways coupled to a waste chamber and to a shared fluid port of the microfluidic cartridge for delivery of a wash solution and a release solution. The liquid handling system delivers a wash fluid through the shared fluid port to wash the set of nucleic acid-magnetic bead samples, captured within the magnetic field, and then delivers a release fluid through the shared fluid port to release a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples. In the specific example, each fluidic pathway is washed sequentially, and the release solution is delivered to each fluidic pathway sequentially to ensure that each lane is provided with substantially equal amounts of wash and release solutions. All waste fluid produced in the specific example of Step S440 pass into the waste chamber coupled to the set of truncated fluidic pathways.

Step S450 recites combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures, which functions to prepare the set of nucleic acid volumes to be detected. For each nucleic acid volume in the set of nucleic acid volumes, Step S450 preferably comprises aspirating an entire volume of the nucleic acid volume from its corresponding fluidic pathway, and transferring the nucleic acid volume to a well containing a molecular diagnostic reagent. Preferably, all nucleic acid volumes in the set of nucleic acid volumes are aspirated and combined with molecular diagnostic reagents simultaneously using a multichannel fluid delivery system; however, each nucleic acid volume in the set of nucleic acid volumes may alternatively be aspirated and combined with molecular diagnostic reagents independently of the other nucleic acid volumes. The molecular diagnostic reagents preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to detect any specific nucleic acid sequence.

In a first variation of Step S450 as shown in FIG. 16A, a nucleic acid volume is aspirated and combined with a molecular diagnostic reagent for a single assay. In the first variation of Step S450, a set of nucleic acid volumes may thus be aspirated simultaneously, and each nucleic acid volume may be transferred to an individual well to be combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures. In the first variation of Step S450, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures may or may not be substantially identical in composition, depending

on the homogeneity of the biological samples used in Step S410; however, the first variation of S450 preferably comprises using identical molecular diagnostic reagents, such that identical molecular diagnostic protocols analyzing identical markers may be performed. Thus, the first variation of 5 Step S450 encompasses running multiple identical tests from a stock biological sample (e.g., a multiplex assay), and run-

ning identical tests using a set of substantially different bio-

logical samples (e.g., from different sources).

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In a second variation of Step S450, as shown in FIG. 16B, 10 the set of nucleic acid volumes is aspirated, and each nucleic acid volume in the set of nucleic acid volumes is combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents. In the second variation of Step S450, the set of molecular diagnostic reagents preferably comprises 15 different molecular diagnostic reagents, such that different molecular diagnostic protocols analyzing different markers may be performed. Thus, the second variation encompasses running multiple substantially different tests using a stock biological sample, and running substantially different tests using substantially different biological samples (e.g., from different sources)

In a specific example of Step S450, a multichannel liquid handling system aspirates approximately 18  $\mu L$  of each of a set of nucleic acid volumes from the microfluidic cartridge used in the specific example of Step S440 using a set of pipette tips, punctures at least one foil seal 195 of at least one assay strip, wherein each well of the at least one assay strip contains molecular diagnostic reagents, and dispenses each aspirated nucleic acid volume into a well of the assay strip. In the 30 specific example of S450, the multichannel liquid handling system then aspirates and dispenses the contents of each well approximately 10 times to reconstitute molecular diagnostic reagents and mix the contents of each well.

Step S460 recites transferring each of the set of nucleic 35 acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all 40 nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-reagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently 45 of the other nucleic acid reagent mixtures. Step S460 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S462, which functions to define at least one truncated fluidic pathway coupled to a detection chamber of a set of 50 detection chambers. Preferably, Step S462 comprises occluding each fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions, thus defining a set of truncated fluidic pathways, each coupled to a detection cham-

In a specific example of Step S460, the multichannel liquid handling subsystem of the specific example of Step S450 transfers a set of nucleic acid-reagent mixtures, each having a volume of approximately 16  $\mu L$ , back to the set of fluidic pathways of the microfluidic cartridge of the specific example 60 of Step S450. Each nucleic acid-reagent mixture in the set of nucleic acid-reagent mixtures is transferred at a rate of 50  $\mu L/$ minute. Manipulation of the elastomeric layer at a subset of a set of occlusion positions by the valve actuation subsystem of the molecular diagnostic module defines a set of 65 truncated fluidic pathways, each coupled to a detection chamber, such that each nucleic acid-magnetic bead sample in the

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set of nucleic acid-magnetic bead samples is isolated within a truncated fluidic pathway of the set of truncated fluidic pathways. In the specific embodiment the occlusion position immediately upstream of the detection chamber and the occlusion position immediately downstream of the detection chamber are normally closed positions. During delivery, the multichannel liquid handling subsystem generates pressure to cause the elastomeric layer at the normally closed positions to deform and allow fluid to flow through the normally closed positions. Once the pressure drops after the detection chamber is filled and the multichannel liquid handing subsystem ceases delivery, the elastomeric layer is configured to overcome the pressure in the channel and recloses, thereby sealing the normally closed positions. The normally closed positions are then compressed using the valve actuation subsystem during thermocycling to prevent pressures generated during a molecular diagnostic assay to cause the normally closed positions to leak. After the molecular diagnostic assay is complete and the occlusion "pins" withdrawn, the normally closed positions allow the samples and amplicons to be trapped within detection chambers, substantially reducing the risk of contamination of the lab or other samples.

Step S470 recites receiving light from the set of nucleic acid-reagent mixtures, and functions to produce emission responses from the set of nucleic acid-reagent mixtures in response to transmission of excitation wavelength light or chemiluminescent effects. Preferably, Step S470 comprises the ability to transmit light including a wide range of wavelengths through a set of excitation filters and through a set of apertures configured to individually transmit light having single or multiple excitation wavelengths onto the set of nucleic acid-reagent mixtures, and receiving light through a set of emission filters, from the set of nucleic acid-reagent mixtures. Step S470 may additionally comprise reflecting light from the set of excitation filters off of a set of dichroic mirrors, and transmitting light through the set of dichroic mirrors to a set of photodetectors. A specific example of Step S470 comprises using the optical subsystem 180 of the system 100 described above to transmit and receive light; however, alternative variations of Step S470 may use any appropriate optical system configured to transmit light at excitation wavelengths toward the set of nucleic acid-reagent mixtures, and to receive light at emission wavelengths from the set of nucleic acid-reagent mixtures.

Step S480 recites generating a set of data based on light received from the set of nucleic acid-reagent mixtures, which functions to produce quantitative and/or qualitative data from the set of nucleic acid-reagent mixtures. Step S480 may further function to enable detection of a specific nucleic acid sequence from the nucleic acid-reagent mixture, in order to identify a specific nucleic acid sequence, gene, or organism. Preferably, Step S480 includes converting electrical signals, produced by a set of photodetectors upon receiving light from the set of nucleic acid-reagent mixtures, into a quantifiable metric; however, S480 may alternatively comprise converting electromagnetic energy, received by a set of photodetectors from the set of nucleic acid-reagent mixtures, into a set of qualitative data. In one variation of Step S480, the set of data may be processed by a processor and rendered on a user interface; however, in other variations of Step S480, the set of data may alternatively not be rendered on a user interface.

The method **400** may further comprise re-running a biological sample S**490** if processing and/or analysis of the biological sample results in less than ideal results. Preferably, Step S**490** occurs if an analysis of a biological sample is indeterminate due to machine or user error. Additionally, Step

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S490 preferably occurs automatically upon detection of a less than ideal result, but may alternatively occur in response to a user prompt.

Embodiments of the method **400** and variations thereof can be embodied and/or implemented at least in part by a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system **100** and one or more portions of the processor **273** and/or the controller **272**. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instructions.

The FIGURES illustrate the architecture, functionality and 20 operation of possible implementations of systems, methods and computer program products according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of 25 code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams 35 and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

As a person skilled in the art will recognize from the  $_{40}$  previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

#### We claim:

- 1. A method for processing and detecting nucleic acids from a biological sample, with a cartridge including a fluidic pathway coupled to a sample port and a reagent port, wherein the method comprises:
  - aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots:
  - dispensing the biological sample into the fluidic pathway through the sample port of the cartridge;
  - moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a subset of the set of pins through the set of slots of the cartridge 60 platform and occluding the fluidic pathway at a subset of occlusion positions to form a first truncated portion of the fluidic pathway for controlling the flow of the biological sample through the fluidic pathway;
  - separating a volume of nucleic acids from the biological 65 sample within the first truncated portion of the fluidic pathway;

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- removing a portion of the volume of nucleic acids from the fluidic pathway through at least one of the reagent port and the sample port of the cartridge;
- combining the portion of the volume of nucleic acids with a molecular diagnostic reagent to produce a nucleic acid-reagent mixture; and
- delivering the nucleic acid-reagent mixture through a second portion of the fluidic pathway to a detection chamher.
- 2. The method of claim 1, further comprising combining the biological sample with a quantity of magnetic beads, to produce a magnetic bead-sample mixture, prior to dispensing the biological sample into the fluidic pathway through the sample port.
- 3. The method of claim 2, wherein combining the biological sample with a quantity of magnetic beads further comprises heating the biological sample with the quantity of magnetic beads.
- 4. The method of claim 2, wherein a supernatant is not removed from the magnetic bead-sample mixture prior to dispensing the biological sample into the fluidic pathway.
- 5. The method of claim 2, wherein the molecular diagnostic module comprises a magnet, and wherein separating a volume of nucleic acids from the biological sample comprises passing the magnet through one of the set of slots of the cartridge platform, thereby providing a magnetic field at the first truncated portion of the fluidic pathway to capture the magnetic bead-sample within the first truncated portion of the fluidic pathway.
- **6**. The method of claim **5**, wherein separating the volume of nucleic acids from the biological sample further comprises dispensing a wash solution through the first truncated portion of the fluidic pathway by a fluid port coupled to the fluidic pathway.
- 7. The method of claim 6, wherein separating the volume of nucleic acids from the biological sample further comprises dispensing a release solution through the fluidic pathway by the fluid port.
- **8**. The method of claim **7**, wherein separating the volume of nucleic acids from the biological sample further comprises heating the first portion of the fluidic pathway to facilitate a pH shift, thus releasing nucleic acids from magnetic beads to produce the volume of nucleic acids.
- 9. The method of claim 1, wherein combining the portion of the volume of nucleic acids with a molecular diagnostic reagent to produce a nucleic acid-reagent mixture comprises aspirating the nucleic acid-reagent mixture from a well and delivering the nucleic acid-reagent mixture into the well multiple times.
- 10. The method of claim 1, wherein delivering the nucleic acid-reagent mixture through a second portion of the fluidic pathway comprises delivering the nucleic acid-reagent mixture through the reagent port of the cartridge.
- 11. The method of claim 1, further comprising generating a set of data based on receiving light from the nucleic acid-reagent mixture through an emission filter.
- 12. The method of claim 11, wherein generating the set of data further comprises detecting a specific nucleic acid sequence from the nucleic acid-reagent mixture within the detection chamber.
- 13. The method of claim 1, wherein delivering the nucleic acid-reagent mixture through a second portion of the fluidic pathway to a detection chamber comprises delivering the nucleic acid-reagent mixture through a second portion of a fluidic pathway of a second cartridge.

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- 14. The method of claim 1, wherein at least one of dispensing, separating, removing, combining, and delivering is automatically re-performed for the biological sample in response to an indeterminate result.
- 15. A method for processing and detecting nucleic acids 5 from a set of biological samples with a cartridge having a set of fluidic pathways defined by an elastomeric layer, the method comprising:
  - combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of nucleic acid-magnetic bead samples;
  - aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam 15 module contacting a set of pins aligned with the set of
  - transferring substantially all of each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of 20 fluidic pathways;
  - moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric 25 layer to occlude at least one fluidic pathway of the set of fluidic pathways at a subset of occlusion positions for controlling a flow through the fluidic pathway; and
  - detecting nucleic acids using a set of detection chambers coupled to the set of fluidic pathways.
- 16. The method of claim 15, wherein the method for processing and detecting nucleic acids from a set of biological samples comprises processing and detecting nucleic acids from identical portions of a stock biological sample, such that all biological samples in the set of biological samples are 35 substantially identical in composition.
- 17. The method of claim 15, wherein combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of nucleic acidnucleic acid-magnetic bead samples.
- 18. The method of claim 15, wherein combining each biological sample of the set of biological samples with a quantity of magnetic beads comprises combining each biological sample of the set of biological samples with a quantity 45 of magnetic beads treated to be at least one of positively charged, magnetic, paramagnetic, and supraparamgnetic.
- 19. The method of claim 15, further comprising producing a set of nucleic acid volumes from the set of nucleic acidmagnetic bead samples, prior to detecting nucleic acids using 50 a set of detection chambers.
- 20. The method of claim 19, wherein producing the set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples comprises:
  - upon passing a magnet through one of the set of slots of the 55 cartridge platform of the molecular diagnostic module, providing a magnetic field spanning a portion of each fluidic pathway in the set of fluidic pathways, thus capturing the set of nucleic acid-magnetic bead samples;
  - dispensing a wash solution into each fluidic pathway of the 60 set of fluidic pathways;
  - dispensing a release solution into each fluidic pathway of the set of fluidic pathways; and
  - heating each fluidic pathway of the set of fluidic pathways to facilitate a pH shift, thus unbinding nucleic acids from 65 magnetic beads to produce the set of nucleic acid volumes.

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- 21. The method of claim 20, wherein dispensing the wash solution and dispensing a release solution comprises dispensing a wash solution and dispensing a release solution into a fluid port, wherein the fluid port is coupled to each fluidic pathway in the set of fluidic pathways.
- 22. The method of claim 21, further comprising coupling the fluid port to an external fluid handling system.
- 23. The method of claim 19, further comprising combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents prior to detecting nucleic acids using a set of detection chambers.
- 24. The method of claim 23, wherein all molecular diagnostic reagents of the set of molecular diagnostic reagents are configured to facilitate identical assays.
- 25. The method of claim 16, further comprising producing a set of nucleic acid volumes from the stock biological sample, and combining the set of nucleic acid volumes with a set of molecular diagnostic reagents, to facilitate running multiple assays from the stock biological sample.
- 26. The method of claim 19, further comprising aspirating the set of nucleic acid volumes from the set of fluidic pathways, wherein aspirating comprises aspirating the set of nucleic acid volumes from a set of reagent ports, wherein each reagent port of the set of reagent ports is coupled to a corresponding fluidic pathway of the set of fluidic pathways.
- 27. The method of claim 26, further comprising transferring each of the set of nucleic acid-reagent mixtures into the corresponding fluidic pathway of the set of fluidic pathways, wherein transferring comprises transferring the set of nucleic acid-reagent mixtures back into the set of reagent ports.
- 28. The method of claim 19, further comprising transmitting light through a set of excitation filters toward the set of nucleic acid-reagent mixtures within the set of detection chambers, and receiving light from the set of nucleic acidreagent mixtures through a set of emission filters.
- 29. A method for processing and detecting nucleic acids within a cartridge having a fluidic pathway including a set of magnetic bead samples further comprises heating the set of 40 occlusion positions defined by an elastomeric layer of the cartridge, the method comprising:
  - aligning the cartridge at a cartridge platform of a molecular diagnostic module, the cartridge platform having a set of slots, and the molecular diagnostic module having a cam module contacting a set of pins aligned with the set of slots and an actuator that provides relative displacement between the cartridge platform and the set of pins;
  - moving the cam module by transitioning the actuator into an extended configuration, thereby displacing a first subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway at a first subset of the set of occlusion positions, thus defining a first truncated fluidic pathway passing through a magnetic field for controlling a flow through the fluidic path-
  - capturing a sample of nucleic acids bound to magnetic beads within the first truncated fluidic pathway, by the magnetic field: and
  - moving the cam module, thereby displacing a second subset of the set of pins through the set of slots of the cartridge platform, and thereby manipulating the elastomeric layer to occlude the fluidic pathway, through the elastomeric layer, at a second subset of the set of occlusion positions, thus defining a second truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads.

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- 30. The method of claim 29, further comprising:
- delivering a wash solution into the second truncated fluidic pathway through a fluid port to facilitate production of a volume of nucleic acids
- delivering the volume of nucleic acids through a reagent <sup>5</sup> port coupled to the fluidic pathway;
- receiving the volume of nucleic acids combined with a volume of molecular diagnostic reagents to produce a nucleic acid-reagent sample;
- occluding the fluidic pathway at a third subset of the set of occlusion positions, thus defining a third truncated fluidic pathway coupled to a detection chamber; and
- delivering the nucleic acid-reagent sample, through the third truncated fluidic pathway, to the detection chamber
- 31. The method of claim 30, wherein delivering a wash solution comprises delivering a wash solution using a syringe pump.

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- 32. The method of claim 30, wherein at least one of delivering the volume of nucleic acids and delivering the nucleic acid-reagent sample comprises delivering using a multichannel pipette head.
- 33. The method of claim 30, further comprising:
- occluding the fluidic pathway at a fourth subset of the set of occlusion positions, thus defining a fourth truncated fluidic pathway containing the sample of nucleic acids bound to magnetic beads and coupled to the fluid port, and
- delivering a release solution into the fourth truncated fluidic pathway through the fluid port to facilitate production of the volume of nucleic acids.
- 34. The method of claim 30, wherein delivering the nucleic acid-reagent sample to the detection chamber comprises delivering the nucleic acid-reagent sample through the reagent port.

\* \* \* \* \*

# EXHIBIT 27

# (12) United States Patent Williams et al.

# (10) Patent No.: US 9,403,165 B2 (45) Date of Patent: \*Aug. 2, 2016

#### -----

### (43) Date 011

(56)

### (58) Field of Classification Search

#### (54) MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS

# ACIDS

# (71) Applicant: **NeuMoDx Molecular, Inc.**, Ann Arbor, MI (US)

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# (73) Assignee: **NeuMoDx Molecular, Inc.**, Ann Arbor, MI (US)

#### (\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35

U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: 14/755,821

(22) Filed: **Jun. 30, 2015** 

#### (65) **Prior Publication Data**

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- (63) Continuation of application No. 13/766,009, filed on Feb. 13, 2013, now Pat. No. 9,101,930.
- (60) Provisional application No. 61/598,240, filed on Feb. 13, 2012, provisional application No. 61/667,606, filed on Jul. 3, 2012.

(51) **Int. Cl. B01L 3/00** (2006.01) **C12Q 1/68** (2006.01)
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## None See application file for complete search history.

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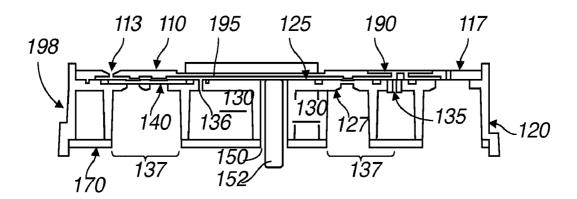
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#### (57) ABSTRACT

A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.

#### 18 Claims, 18 Drawing Sheets



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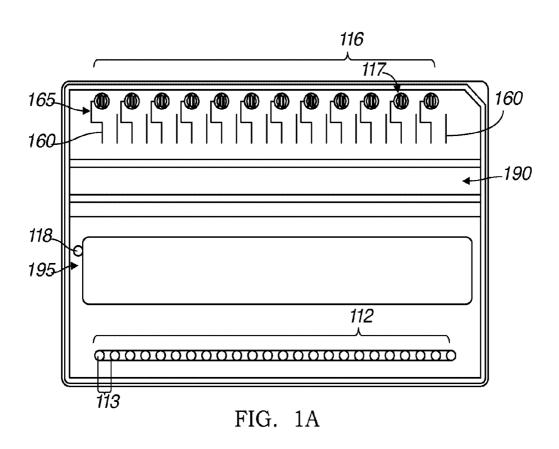
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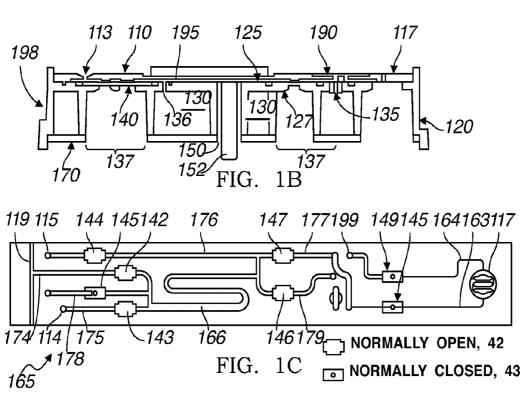
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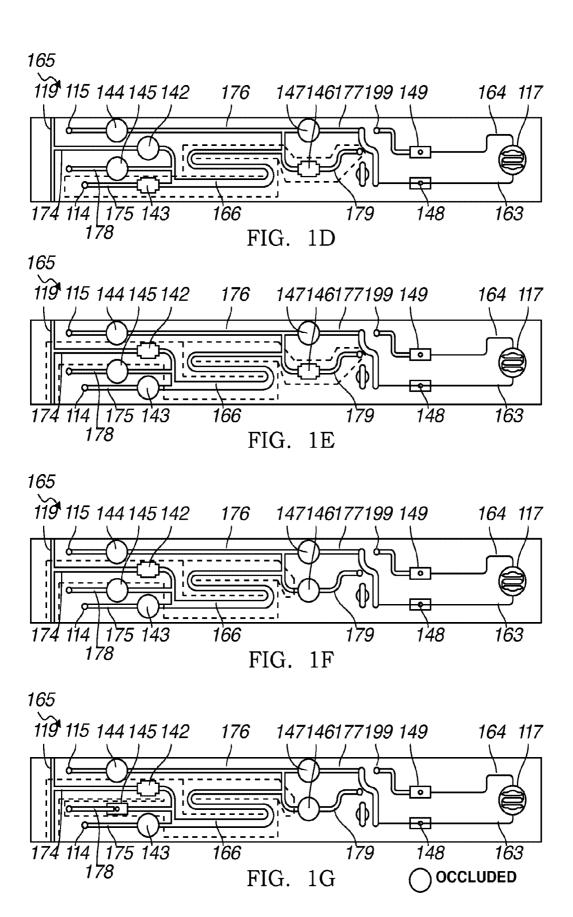
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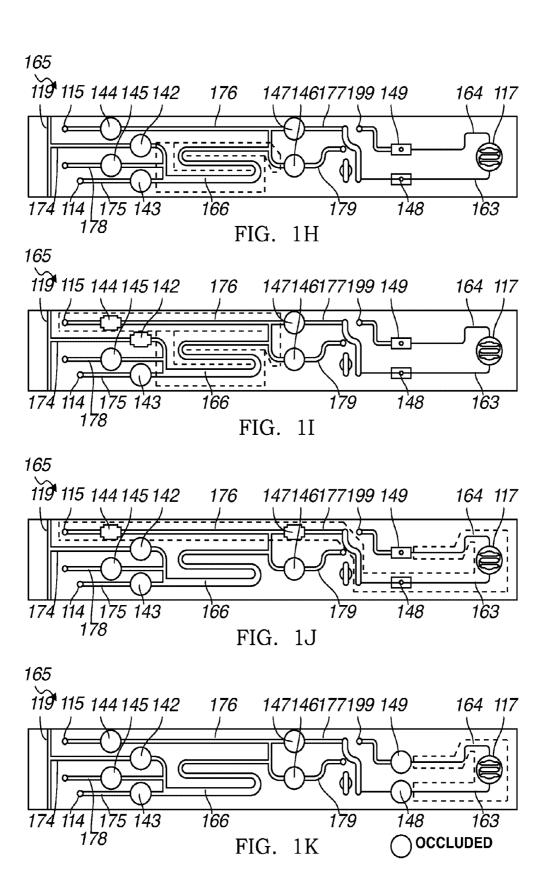
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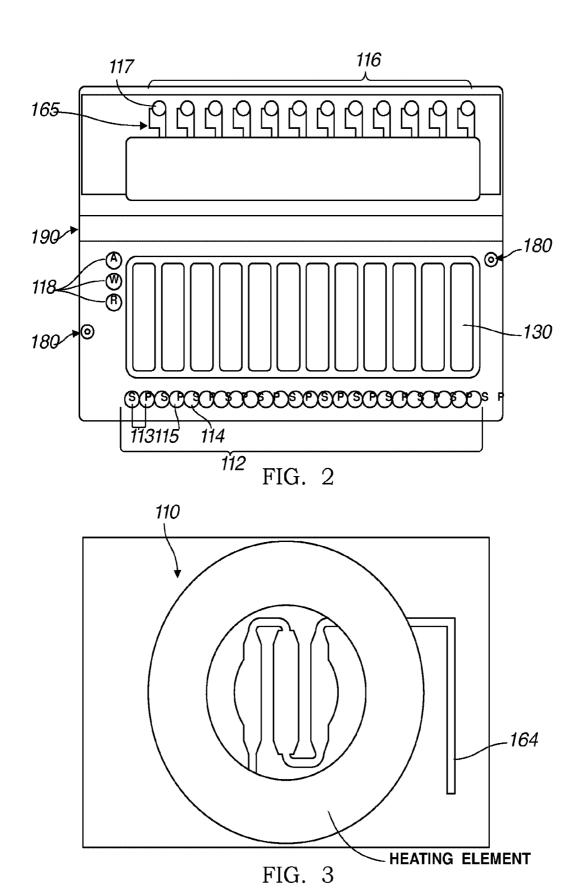
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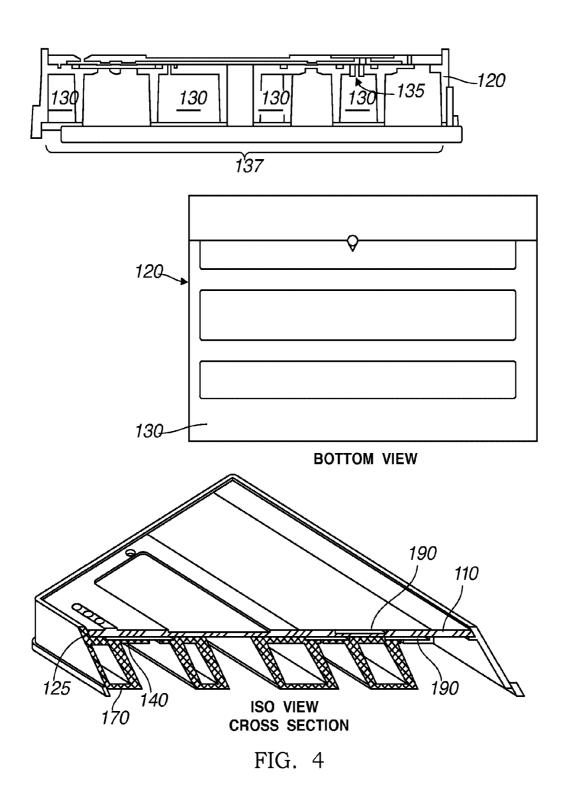
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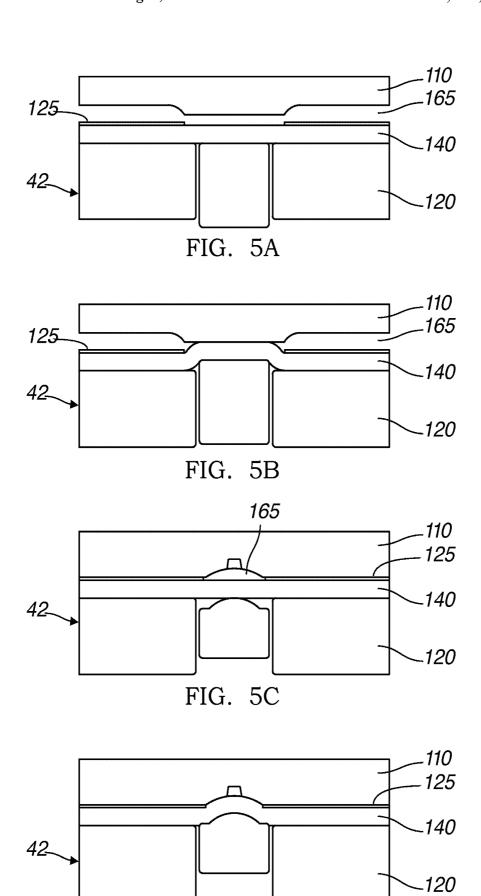


FIG. 5D

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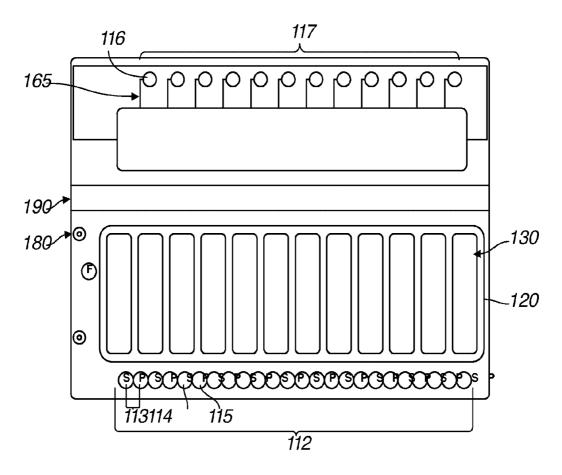


FIG. 6A

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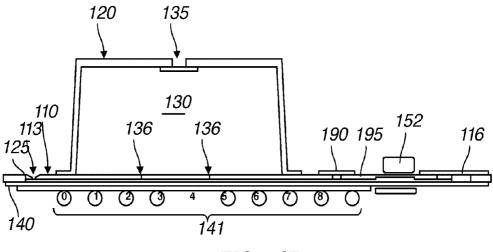


FIG. 6B

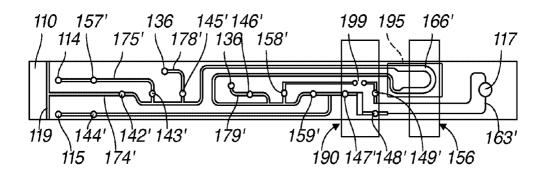


FIG. 6C

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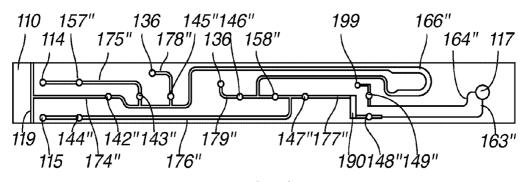
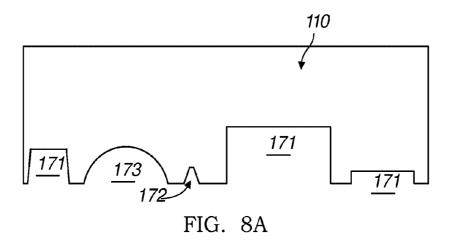


FIG. 7



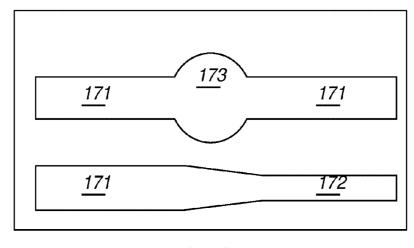
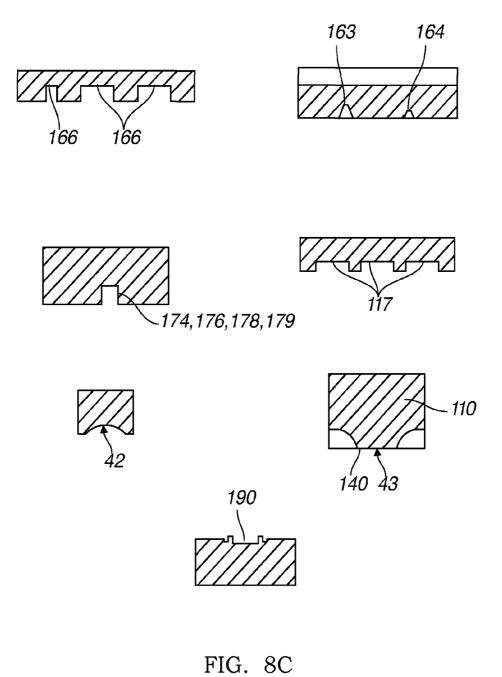


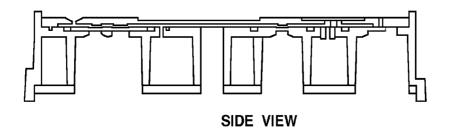
FIG. 8B

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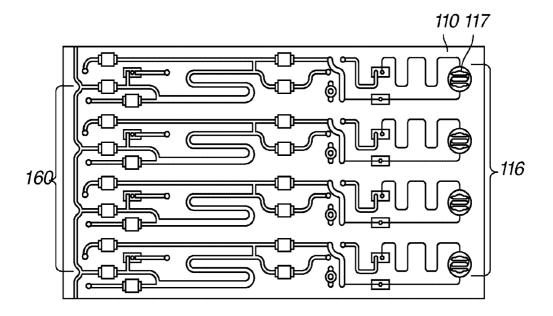


FIG. 9
TOP VIEW

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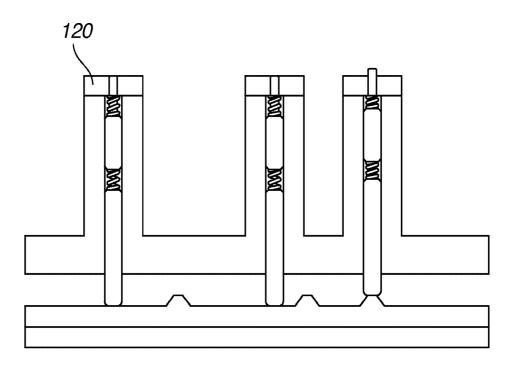


FIG. 10A

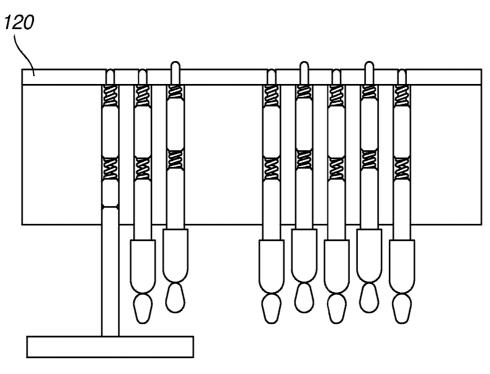
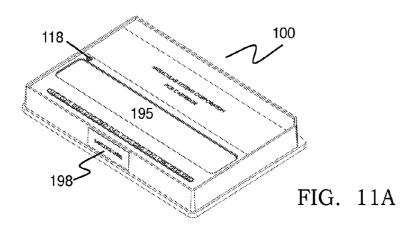
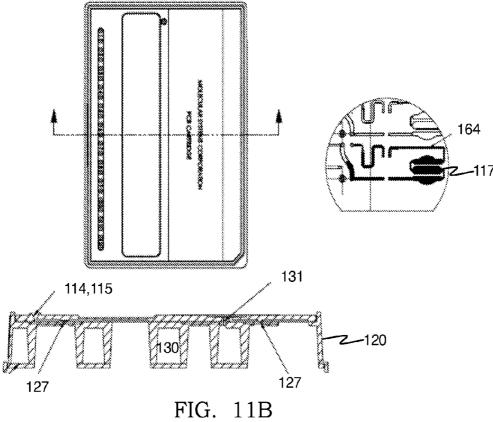


FIG. 10B

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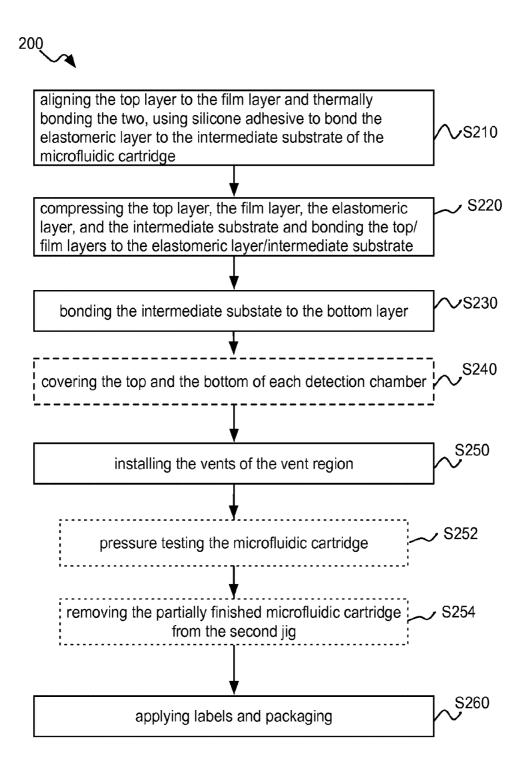
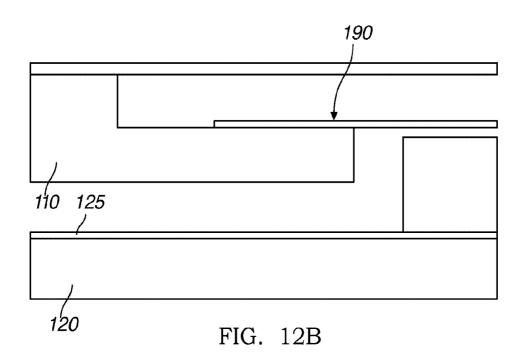


FIG. 12A

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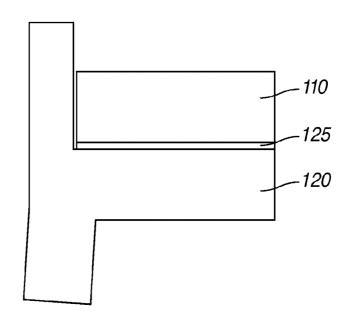
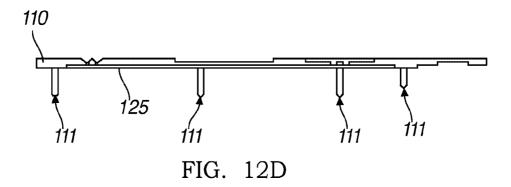


FIG. 12C

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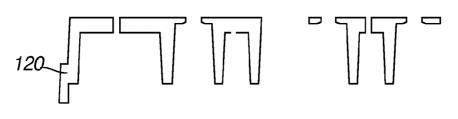


FIG. 12E

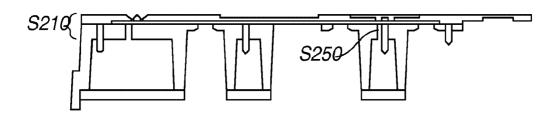


FIG. 12F

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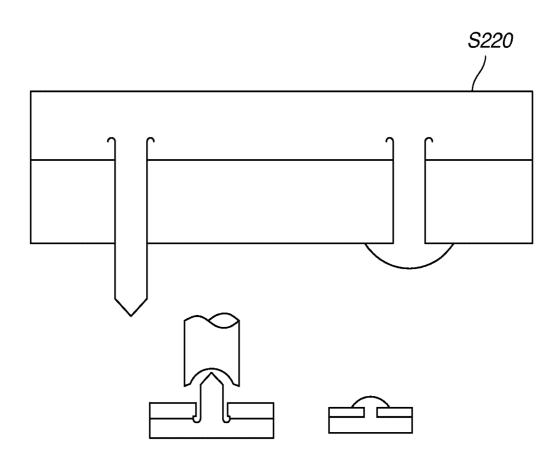
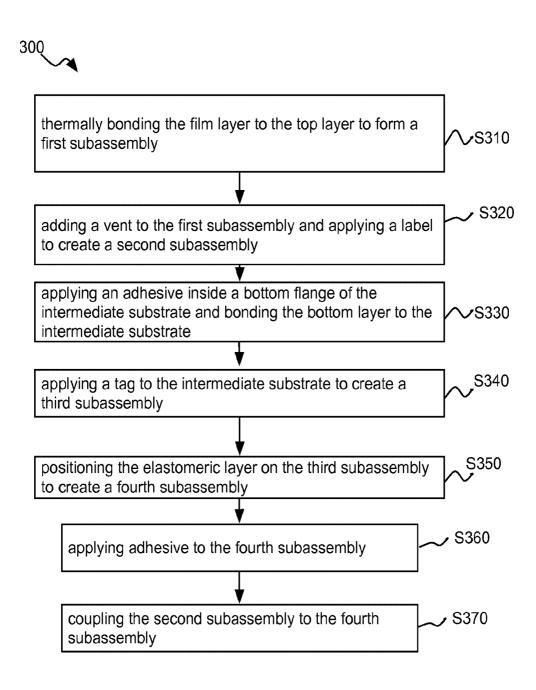


FIG. 12G

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## 1

## MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS

# CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 13/766,009 (now U.S. Pat. No. 9,101,930), filed 13 Feb. 2013, which claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are all incorporated in their entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved microfluidic cartridge for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a laboratory discipline that has 25 developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diag- 30 nostic use in healthcare and other fields requiring nucleic acid analysis. Molecular diagnostic analysis of biological samples can include the detection and/or monitoring of one or more nucleic acid materials present in the specimen. The particular analysis performed may be either qualitative and/or quantitative. Methods of analysis may involve isolation, purification, and amplification of nucleic acid materials, and polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecular diagnostic techniques are also labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are often specific to certain nucleic acid types and not applicable across multiple acid types. Due to these and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for improved devices 50 for processing and amplifying nucleic acids. Thus, there is a need in the molecular diagnostics field to create an improved microfluidic cartridge to facilitate processing and detecting of nucleic acids. This invention provides such a microfluidic cartridge.

## BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1C depict an embodiment of a microfluidic cartridge (top and side views) and an embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. 1D-K depict an example embodiment of subsets of occlusion positions defining truncated portions of a fluidic pathway;

FIG. 2 depicts an alternative embodiment of a microfluidic 65 cartridge (top view) showing individual waste chambers located on the top of cartridge and multiple fluid ports;

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FIG. 3 depicts an alternative embodiment of a detection chamber of the microfluidic cartridge (top view) and a heating element configured to heat the detection chamber;

FIG. 4 depicts an embodiment of a waste chamber of the microfluidic cartridge;

FIGS. 5A-5D depict embodiments of the elastomeric layer of the microfluidic cartridge, in open and occluded configurations:

FIGS. **6A-6**C depict an alternative embodiment of a microfluidic cartridge (top and side views) and an alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIG. 7 depicts another alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. **8**A and **8**B depict schematics of microfluidic channel cross sections;

FIG. 8C depicts specific embodiments of microfluidic channel cross sections;

FIG. 9 depicts an embodiment of the microfluidic cartridge with twelve fluidic pathways (four of which are shown);

FIGS. 10A and 10B depict embodiments of occlusion of fluidic pathways with the elastomeric layer and a valving mechanism:

FIGS. 11A and 11B depict an embodiment of the microfbuildic cartridge;

FIGS. 12A-12G depict an example manufacturing method for an embodiment of the microfluidic cartridge; and

FIG. 13 depicts an alternative example manufacturing method for an embodiment of the microfluidic cartridge.

# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

## 1. Microfluidic Cartridge

As shown in FIGS. 1A-1C, an embodiment of a microflu- $\,$  40  $\,$  idic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample portreagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140. In other embodiments, the microfluidic cartridge 100 may further comprise a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber 130. Furthermore, the top layer 110 of the microf-55 luidic cartridge 100 may further comprise a shared fluid port 118, a vent region 190, and a heating region 195, such that each fluidic pathway 165 in the set of fluidic pathways 160 is fluidically coupled to a sample port-reagent port pair 113, the shared fluid port 118, the waste chamber 130, and a detection chamber 117, comprises a capture segment 166 configured to pass through the heating region and the magnetic field, and is configured to pass through the vent region 190 upstream of the detection chamber 117. Each fluidic pathway 165 thus functions to receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 165. As configured, the microfluidic cartridge 100 can be used to facilitate molecular diag-

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nostic processes and techniques, and preferably conforms to microtiter plate dimensional standards. Alternatively, the microfluidic cartridge 100 may be any appropriate size. In a specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample 5 containing nucleic acids.

#### 1.1 Microfluidic Cartridge—Top Layer

The top layer 110 of an embodiment of the microfluidic cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), 10 such that a sample containing nucleic acids, passing through the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques. Preferably, the top layer 110 is composed of a polypropylene-based polymer, but the top 20 layer 110 may alternatively be composed of any appropriate material (e.g. cyclic olefin polymer). In a specific embodiment, the top layer 110 is composed of 1.5 mm thick polypropylene produced by injection molding, with a glass transition temperature between 136 and 163° C. The top layer 110 may 25 alternatively be composed of any appropriate material, for example, a polypropylene based polymer. As shown in FIGS. 1B and 1C, the top layer 110 preferably comprises a set of sample port-reagent port pairs 112, a fluid port 118, a vent region 190, a heating region 195 crossing a capture segment 30 166 of a fluidic pathway 165, and a set of detection chambers

Each sample-port-reagent port pair 113 of an embodiment of the top layer 110 comprises a sample port 114 and a reagent port 115. The sample port 114 functions to receive a volume 35 of a sample fluid potentially containing the nucleic acids of interest for delivery of the volume of fluid to a portion of a fluidic pathway 165 coupled to the sample port-reagent port pair 113. In a specific embodiment, the volume of a sample fluid is a biological sample with magnetic beads for nucleic 40 acid isolation; however, the volume of fluid comprising a sample fluid may alternatively be any appropriate fluid containing a sample with nucleic acids. Preferably, each sample port 114 is isolated from all other sample ports, in order to prevent cross-contamination between samples of nucleic 45 acids being analyzed. Additionally, each sample port 114 is preferably of an appropriate geometric size and shape to accommodate a standard-size pipette tip used to deliver the volume of a sample fluid without leaking. Alternatively, all or a portion of the sample ports 114 are configured to be coupled 50 to fluid conduits or tubing that deliver the volume of a sample

Each sample-port reagent port pair 113 of an embodiment of the top layer 110 also comprises a reagent port 115, as shown in FIG. 1A. The reagent port 115 in a sample port- 55 reagent port pair 113 functions to receive a volume of fluid comprising a reagent used in molecular diagnostics, for delivery of the volume of fluid comprising a reagent to a portion of a fluidic pathway 165 coupled to the sample port-reagent port pair 113. In a specific embodiment, the volume of fluid comprising a reagent used in molecular diagnostics is a sample of reconstituted molecular diagnostic reagents mixed with nucleic acids released and isolated using the microfluidic cartridge 100; however, the volume of fluid comprising a reagent used in molecular diagnostics may alternatively be 65 any appropriate fluid comprising reagents used in molecular diagnostics. Preferably, each reagent port 115 is isolated from

all other reagent ports, in order to prevent cross-contamination between samples of nucleic acids being analyzed. Additionally, each reagent port 115 is preferably of an appropriate geometric size to accommodate a standard-size pipette tip used to deliver the volume of fluid comprising a reagent used in molecular diagnostics. Alternatively, all or a portion of the reagent ports 115 are configured to be coupled to fluid conduits or tubing that deliver the volume of fluid comprising a reagent used in molecular diagnostics.

Preferably, the set of sample port-reagent port pairs 112 is located near a first edge of the top layer 110, such that the configuration of the sample port-reagent port pairs 112 functions to increase accessibility, for instance, by a pipettor delivering fluids to the microfluidic cartridge 100. In one specific example, the microfluidic cartridge 100 is configured to be aligned within a module, with the set of sample portreagent port pairs 112 accessible outside of the module, such that a multichannel pipette head can easily access the set of sample port-reagent port pairs 112. Preferably, as shown in FIG. 1A, the set of sample port-reagent port pairs 112 is configured such that the sample ports 114 and the reagent ports 115 alternate along the first edge of the top layer 110. In an alternative embodiment, the set of sample port-reagent port pairs 112 may not be located near an edge of the top layer 110, and may further not be arranged in an alternating fash-

The fluid port 118 of the top layer 110 of the microfluidic cartridge functions to receive at least one of a wash fluid, a release fluid, and a gas used in a molecular diagnostic procedure, such as PCR. In an embodiment, the wash fluid, the release fluid, and/or the gas are common to all samples being analyzed during a run of the diagnostic procedure using the microfluidic cartridge 100; in this embodiment, as shown in FIG. 1A, the fluid port 118 is preferably a shared fluid port, fluidically coupled to all fluidic pathways 165 coupled to the sample port-reagent port pairs 112, and configured to deliver the same wash fluid, release fluid, and/or gas through the shared fluid port. Alternatively, as shown in FIG. 2, the top layer may comprise more than one fluid port 118, configured to deliver different wash fluids, release fluids, and/or gases to individual or multiple fluidic pathways 165 coupled to the set of sample port-reagent port pairs 112.

Preferably, the fluid port 118 is located along an edge of the microfluidic cartridge 100, which functions to increase accessibility to the fluid port by a system delivering fluids to the fluid port 118. In a specific embodiment, as shown in FIG. 1A, the fluid port is located approximately midway along an edge of the microfluidic cartridge 100, different from the edge along which the set of sample port-reagent port pairs 112 is located. Alternatively, the fluid port 118 may not be located along an edge of the microfluidic cartridge 100. Additionally, the fluid port 118 is preferably configured to be coupled to a syringe pump for fluid delivery; however, the fluid port 118 may alternatively configured to couple to any appropriate system for fluid delivery. Preferably, the wash fluid is a wash buffer for washing bound nucleic acid samples (i.e. nucleic acids bound to magnetic beads), the release fluid is a reagent for releasing bound nucleic acids samples from the magnetic beads, and the gas is pressurized air for moving fluids and demarcating separate reagents. Alternatively, the wash fluid, release fluid, and gas may be any appropriate liquids or gases used to carry out a molecular diagnostic procedure.

The heating region 195 of the top layer 110 functions to accommodate and position a heating element relative to elements of the microfluidic cartridge 100. The heating element preferably heats a defined volume of fluid and the magnetic beads, which has traveled through the microfluidic cartridge

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100, according to a specific molecular diagnostic procedure protocol (e.g. PCR protocol), and is preferably an element external to the microfluidic cartridge 100; alternatively, the heating element may be integrated with the microfluidic cartridge and/or comprise a thermally conductive element inte-5 grated into the microfluidic cartridge 100. The heating region 195 is preferably a recessed fixed region of the top layer 110, downstream of the sample port-reagent port pairs 112, as shown in FIGS. 1A and 1B. Alternatively, the heating region may not be fixed and/or recessed, such that the heating region 10 195 sweeps across the top layer 110 of the microfluidic cartridge 100 as the heating element is moved. The microfluidic cartridge 100 may altogether omit the heating region 195 of the top layer 110, in alternative embodiments using alternative processes (e.g. chemical methods) for releasing nucleic 15 acids from nucleic acid-bound magnetic beads.

The vent region 190 of an embodiment of the top layer 110 functions to remove unwanted gases trapped within a fluidic pathway 165 of the microfluidic cartridge, and may additionally function to position a defined volume of fluid within a 20 fluidic pathway 165 of the microfluidic cartridge. The vent region 190 is preferably located downstream of the heating region 195 in an embodiment where the heating region 195 is fixed on the top layer 110 of the microfluidic cartridge 100, but alternatively may be located at another appropriate posi- 25 tion on the top layer 110 such that unwanted gases are substantially removed from the microfluidic cartridge 100 during analysis. The top layer 110 may alternatively comprise more than one vent region 190 located at appropriate positions in the top layer 110. Preferably, as shown in FIGS. 1A and 1B, 30 the vent region 190 is a recessed region in the top layer 110, and further comprises a film covering the vent region 190. Preferably, the film covering the vent region 190 is a gaspermeable but liquid-impermeable film, such that unwanted gases may be released from the microfluidic cartridge 100, 35 but fluids remain within the microfluidic cartridge 100 and flow to the point of contacting the film. This functions to remove unwanted gases and position a defined volume of fluid within a fluidic pathway 165 of the microfluidic cartridge. In a specific embodiment, the film covering the vent 40 region is a hydrophobic porous polytetrafluoroethylenebased material, synthesized to be gas-permeable but liquidimpermeable. Alternatively, the film covering the vent region may be gas and liquid permeable, such that unwanted gases and liquids are expelled from the microfluidic cartridge 100 45 through the vent region 190. Other alternative embodiments of the microfluidic cartridge 100 may altogether omit the vent region.

The set of detection chambers 116 of an embodiment of the top layer no functions to receive a processed nucleic acid 50 sample, mixed with molecular diagnostic reagents, for molecular diagnostic analysis. Preferably, the set of detection chambers 116 is located along an edge of the top layer 110, opposite the edge along which the set of sample port-reagent port pairs 112 is located, which allows sample fluids dis- 55 pensed into the microfluidic cartridge 100 to be processed and mixed with molecular diagnostic reagents on their way to a detection chamber 117 of the set of detection chambers 116 and facilitates access to the detection chambers by external elements performing portions of a molecular diagnostics pro- 60 tocol (e.g. heating and optics systems). Alternatively, the set of detection chambers 116 may not be located along an edge of the top layer 110. In a first variation, as shown in FIGS. 1A and 11B, each detection chamber 117 in the set of detection chambers comprises a serpentine-shaped channel 16 for 65 facilitating analysis of a solution of nucleic acids mixed with reagents. In the first variation, three portions of the serpen6

tine-shaped channel 16 are preferably wide and shallow to facilitate heating, and are interconnected by two narrow portions, which function to increase fluid flow resistance and reduce the proportion of nucleic acid not contained within the detection area. The first variation functions to facilitate filling of the set of detection chambers in a manner that reduces the potential for trapped air bubbles, to facilitate rapid molecular diagnostic techniques, and to comply with current imaging technologies. In a specific example of the first variation, each serpentine-shaped channel 16 is injected molded into the top layer 110 of the microfluidic cartridge 100, and the three interconnected portions of the serpentine-shaped channel 16 are each 1600 µm wide by 400 µm deep.

In a second variation, each detection chamber 117 in the set of detection chambers has a depth between 0.400 mm and 1.00 mm, and a diameter between 3.50 mm and 5.70 mm, to provide a volumetric configuration that facilitates reaction efficiency. In a specific example of the second variation, each detection chamber 117 in the set of detection chambers 116 is configured to contain a total volume of 10 uL, and has a depth of 0.80 mm and a diameter of 3.99 mm; however, in alternative embodiments, each detection chamber 117 in the set of detection chambers 116 may be configured to contain a total volume less than or greater than 10 uL.

Preferably, as shown in FIGS. 1A and 1B, the lower regions of each detection chamber 117 in the set of detection chambers 116 includes a PCR compatible film that is thin, to facilitate efficient thermocycling, and has low autofluorescence, to facilitate light-based molecular diagnostic assays performed at the set of detection chambers 116. The PCR compatible film is preferably composed of a polypropylene based polymer thermally bonded to the bottom of the top layer, but may alternatively be composed of any appropriate PCR-compatible material and bonded in any fashion. In one specific variation, the PCR compatible film is a cyclic olefin polymer (COP) film, thermally bonded to the top layer 110, with a glass transition temperature suitable for a molecular diagnostic protocol. In one alternative embodiment, depending on the configuration of imaging, heating, and/or cooling elements external to the microfluidic cartridge 100, the top and/or bottom of the detection chambers 117 in the set of detection chambers 116 may be entirely formed of a clear or transparent material (e.g. glass or plastic) allowing transmission of light. In a variation of this alternative embodiment, lensing, other optical components, or additional structures may also be incorporated into the detection chambers, to facilitate light transmission and/or focusing. In the variation of the alternative embodiment, a lens may be manufactured (e.g. injection molded) directly to form a surface of a detection chamber 117.

In the embodiment of the set of detection chambers 116 that includes a PCR compatible film, the PCR compatible film may further include a thermally conductive component, which functions to transfer heat from a heating element to the detection chamber. Depending on the position of the heating element(s) relative to the microfluidic cartridge 100 during analysis, the thermally conductive component of the PCR compatible film may be integrated with just the upper region of each detection chamber, just the lower region of each detection chamber, or both the upper and lower regions of each Detection chamber. The thermally conductive component of the PCR compatible film may comprise a wire mesh with a substantially small wire diameter, as shown in FIG. 3, thermally conductive particles distributed through the PCR compatible film (in a manner that still allows for optical clarity), or any other appropriate thermally conductive component (e.g. thermally conductive beads integrated into the

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PCR compatible film). The region laterally around the detection chamber may also further include one or more heat-transfer elements or air channels speed heat dissipation. Alternatively, a detection chamber 117 in the set of detection chambers 116 may not include a PCR compatible film with a 5 thermally conductive component. Preferably, each detection chamber 117 is heated using a diced silicon wafer with conductive channels flip-chip bonded to a detection chamber to provide resistive heating; however, each detection chamber 117 may alternatively be heated using any appropriate heating device or method, and may be assembled using any appropriate method.

Preferably, each detection chamber 117 in the set of detection chambers 116 is thermally isolated from all other detection chambers, in order to prevent contamination of data from 15 a detection chamber 117 due to heat transfer from other detection chambers in the set of detection chambers 116. In one embodiment, each detection chamber 117 of the set of detection chambers 116 is spaced far from adjacent detection chambers to limit thermal crosstalk. In another alternative 20 embodiment, the top layer 110 may comprises slots between adjacent detection chambers to separate the detection chambers with an air gap. In one variation, thermal isolation is achieved by surrounding the side walls of each detection chamber 117 with a thermally insulating material, such as an 25 insulating epoxy, putty, filler, or sealant. In another variation, the thermally insulating material has a low density, which functions to reduce heat transfer from other detection chambers. In vet another variation, thermal isolation is achieved by geometrically separating or displacing the detection cham- 30 bers relative to each other within the top layer 110 of the microfluidic cartridge 100, such that heat transfer between detection chambers is hindered.

Preferably, each detection chamber 117 in the set of detection chambers 116 is also optically isolated from all other 35 detection chambers, in order to prevent contamination of data from a detection chamber 117 due to light transfer from other detection chambers in the set of detection chambers 116. Preferably, optical isolation is achieved with detection chambers having substantially vertical walls, and separating each 40 detection chamber 117 in the set of detection chambers from each other. However, in one variation, the sidewalls of each detection chamber 117 in the set of detection chambers 116 are either composed of or surrounded by a material with low autofluorescence and/or poor optical transmission properties 45 to achieve optical isolation. In another variation, the sidewalls of each detection chamber 117 are surrounded by an optically opaque material, thus allowing transmission of light to a detection chamber 117 through only the top and bottom regions of the detection chamber 117. Alternatively, the 50 microfluidic cartridge 100 may not further comprise any provisions for optical isolation of each detection chamber 117 in the set of detection chambers 116, aside from constructing the set of detection chambers 116 with a material having low

Additionally, each detection chamber 117 in the set of detection chambers 116 may be further optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical detection, and facilitate filling in a manner that limits bubble generation. Alternatively each 60 detection chamber 117 in the set of detection chambers 116 may not be optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical detection, and/or facilitate filling in a manner that limits bubble generation.

The top layer 110 of the microfluidic cartridge 100 may further comprise a set of cartridge-aligning indentations 180,

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which function to align the microfluidic cartridge 100 as it moves through an external module. As shown in FIG. 2 the set of cartridge-aligning indentations 180 are preferably located such that they do not interfere with any ports 112, 118, the heating region, 195, the vent region 190, and/or the set of detection chambers 116. In an embodiment, the top layer 110 of the microfluidic cartridge preferably comprises at least four cartridge-aligning indentations, located at points on the periphery of the top layer 110, and the cartridge-aligning indentations are configured to be recessed regions configured to mate with alignment pins in a system external to the microfluidic cartridge 100. Alternatively, the cartridge-aligning indentations may be grooves, such that the microfluidic cartridge 100 accurately slides into position along the grooves within a system external to the microfluidic cartridge 100. In yet another alternative embodiment, the set of cartridgealigning indentations 180 may be any appropriate indentations that allow for positioning of the microfluidic cartridge 100 within an external system. However, the microfluidic cartridge 100 may altogether omit the set of cartridge-aligning indentations 180, and rely upon other features of the microfluidic cartridge 100 to facilitate alignment.

1.2 Microfluidic Cartridge—Intermediate Substrate

As shown in FIG. 1B, an embodiment of the microfluidic cartridge also comprises an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer 110 by a film layer 125, configured to form a waste chamber 130. The intermediate substrate 120 functions to serve as a substrate to which layers of the microfluidic cartridge may be bonded, to provide guides for the valve pins, and to provide a waste chamber volume into which a waste fluid may be deposited. Preferably, the depth of the intermediate substrate 120 provides a waste chamber volume adequate to accommodate the volume of waste fluids generated within the microfluidic cartridge 100. Additionally, the depth of the intermediate substrate 120 provides a low profile for the microfluidic cartridge 100 to facilitate movement throughout a compact molecular diagnostic system. Preferably, the intermediate substrate 120 of the microfluidic cartridge 100 is also configured such that the footprint of microfluidic cartridge 100 adheres to microtiter plate standards, to facilitate automated handling of the microfluidic cartridge 100. The intermediate substrate 120 is preferably composed of a low-cost, structurally stiff material, such as polypropylene. However, similar to the top layer 120, the intermediate substrate may be alternatively composed of a structurally stiff material with low autofluorescence, such that the intermediate substrate 120 does not interfere with sample detection by fluorescence techniques, and an appropriate glass transition temperature for PCR techniques. In one variation of this alternative embodiment, the intermediate substrate 120 is composed of a cyclic olefin polymer (COP), produced by injection molding, with a glass transition temperature between 136 and 163° C. In yet another alternative embodi-55 ment, the intermediate substrate 120 may be composed of any appropriate material, for example, a polycarbonate based

Preferably, the intermediate substrate 120 of the microfluidic cartridge 100 is coupled to the top layer 110 and partially separated from the top layer 110 by a film layer 125. The film layer 125 functions to isolate individual fluidic pathways 165 of the microfluidic cartridge, to prevent leakage, to provide an appropriate environment for sample processing and conducting a molecular diagnostic protocol, and to provide access between a microfluidic channel (of a fluidic pathway 165) above the film layer 125 and elements below the film layer 125 (e.g. waste chamber and/or fluidic pathway occluder).

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Preferably, the film layer is a polypropylene (PP) with an appropriate glass transition temperature, such that it is PCR compatible and thermally bondable to the top layer 110; however, the film layer may alternatively be any appropriate material. In a specific embodiment, the film layer 125 is a 5 polypropylene film between 30 and 100 microns thick and die cut to produce openings at a set of occlusion positions, to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer 125 and elements below the film layer 125. In this specific embodiment, the openings are slightly oversized prior to assembly, in order to allow for constriction during assembly (due to thermal and pressure effects) and to provide higher tolerance during assembly of microfluidic cartridge layers. Alternatively, the film layer is any appropriate material such that it substantially isolates 15 individual fluidic pathways, and is easily processable to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer and elements below the film layer 125.

Preferably, the top layer 110, the film layer 125, and the 20 intermediate substrate are bonded together, such that the top layer 110, film layer, 125, and intermediate substrate form a bonded unit with a hermetic seal to prevent fluid leakage. A hermetic seal is preferably formed using a silicone rubber layer coupled to the film layer 125, but may alternatively be 25 formed using an alternative material or method. In a specific embodiment, a hermetic seal formed using a silicone rubber layer is only required at locations of openings within the film layer (e.g., at locations where an external occluder interacts with the microfluidic cartridge). Preferably, in an embodi- 30 ment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially identical materials (e.g. polypropylene), at least one of thermal bonding, adhesives, and ultrasonic welding are used to coupled the layers 110, 125, 120 together. In an embodiment where the 35 top layer 110, the film layer 125, and the intermediate substrate 120 are substantially different materials—a combination of thermal bonding methods and adhesives may be used to bond the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 40 together. In an alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 may be thermally bonded together in a single step. In yet another alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 45 120 may alternatively be modular, in applications where a portion of the microfluidic cartridge 100 is partially reusable (e.g. in an application where the waste chamber may be discarded after use, but the top layer and film may be reused). In yet another alternative embodiment, the top layer 110, the 50 film layer 125, and the intermediate substrate 120 may only be partially bonded, such that a molecular diagnostic system, into which the microfluidic cartridge 100 is loaded, is configured to compress the top layer 110, the film layer 125, and the intermediate substrate 120 together, preventing any fluid 55 leakage.

As shown in FIG. 1B, the intermediate substrate 120 of an embodiment of the microfluidic cartridge 100 is configured to form a waste chamber 130, which functions to receive and isolate waste fluids generated within the microfluidic cartridge 100. The waste chamber 130 is preferably continuous and accessible by each fluidic pathway 165 of the microfluidic cartridge 100, such that all waste fluids generated within the microfluidic cartridge 100 are deposited into a common waste chamber; however, each fluidic pathway 165 of the 65 microfluidic cartridge 100 may alternatively have its own corresponding waste chamber 130, such that waste fluids

cartridge 100 are isolated from waste fluids generated within other fluidic pathways 165 of the microfluidic cartridge 100. In a specific embodiment of the microfluidic cartridge 100 with a continuous waste chamber, the waste chamber has a volumetric capacity of approximately 25 mL; however, the waste chamber 130 of another embodiment may have a different volumetric capacity. The intermediate substrate 120 further comprises a waste vent 135, which provides access between a microfluidic channel of a fluidic pathway 165 above the film layer 125 and the waste chamber 130. Preferably, the intermediate substrate 130 comprises more than one waste inlet 136, such that the waste chamber is accessible at more than one location along a fluidic pathway 165 through

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generated within a fluidic pathway 165 of the microfluidic

120 may include a single waste inlet 136, such that all waste fluids generated within the microfluidic cartridge 100 are configured to travel through the single waste inlet 136 into the waste chamber 130. Also, as shown in FIG. 1B, the intermediate substrate 120 may comprise a waste vent 131, such that the waste chamber 130 is vented to prevent pressure build up in the waste chamber as waste fluid is added.

the waste inlets 136. Alternatively, the intermediate substrate

As shown in FIGS. 1B and 4, the waste chamber 130 formed by the intermediate substrate 120 preferably has a corrugated surface 137, such that the waste chamber 130 is not only configured to receive and isolate a waste fluid, but also functions to 1) provide structural stability for the microfluidic cartridge 100 and 2) allow elements external to the microfluidic cartridge 100 to enter spaces formed by the corrugated surface 137, for greater accessibility to elements of the microfluidic cartridge 100. Also shown in FIGS. 1B and 4, each of the ridges in the corrugated surface 137 may not have the same dimensions, as a result of the locations of elements within and external to the microfluidic cartridge 100. In an embodiment of the waste chamber 130 with a corrugated surface 137, at least two ridges of the corrugated surface 137 are preferably the same height, such that the microfluidic cartridge 100 sits substantially level on a flat base. In an alternative embodiment, all ridges of the corrugated surface 137 of the waste chamber 130 are identical, for structural symmetry, and in yet another embodiment, the waste chamber 130 may not have a corrugated surface 137.

In one preferred embodiment, the intermediate substrate 120 of the microfluidic cartridge 100 further comprises a set of valve guides, which function to direct a series of external pins or other indenters through the valve guides at a set of occlusion positions 141, thus affecting flow through a microfluidic channel of a fluidic pathway 165 at the set of occlusion positions 141. The set of valve guides 127 may also function to facilitate alignment of the microfluidic cartridge 100 within an external molecular diagnostic module. In a first embodiment, as shown in FIG. 1B, the set of valve guides 127 comprises holes within the intermediate substrate 120 at the set of occlusion positions 141, with sloped edges configured to direct a pin or indenter through the holes. In the first embodiment, the set of valve guides 127 may be produced in the intermediate substrate 120 by injection molding, or may alternatively be produced by drilling, countersinking, chamfering, and/or beveling. In another embodiment, the set of valve guides 127 comprises grooves with holes, such that a pin or indenter is configured to travel along a groove and through a hole that defines the valve guide. In a simplified alternative variation, the set of valve guides 127 may comprise holes through the intermediate substrate 120, wherein the holes do not have sloped edges. In yet another simplified alternative variation, the set of valve guides 127 may comprise a slot configured to provide access to the elastomeric

11 layer 140 by a group of occluding objects (e.g. pins or indenters), rather than a single occluding object.

1.3 Microfluidic Cartridge—Elastomeric and Bottom Layers As shown in FIGS. 1B and 5A-5D, an embodiment of the microfluidic cartridge 100 also comprises an elastomeric 5 layer 140 partially situated on the intermediate substrate 120, which functions to provide a deformable substrate that, upon deformation, occludes a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 at an occlusion position of a set of occlusion positions 141. Preferably, 10 the elastomeric layer 140 comprises an inert, liquid impermeable material, of an appropriate thickness, that can be heated to temperatures encountered during manufacturing and/or specified in a molecular diagnostic protocol, without substantial damage (i.e. compromised surface and/or loss of 15 mechanical robustness) and is chemically compatible with a PCR assay. Preferably, the elastomeric layer 140 is noncontinuous, such that portions of the elastomeric layer 140 are positioned relative to the intermediate substrate 120 in a manner that directly covers holes provided by the set of valve 20 guides 127. Alternatively, the elastomeric layer 140 is a continuous layer, spanning a majority of the footprint of the microfluidic cartridge 100 while covering holes provided by the set of valve guides 127. In a specific embodiment, the elastomeric layer 140 comprises 500 micron thick strips of a 25 low-durometer silicone that can be heated to at least 120° C. without substantial damage, which are bonded to a portion of the intermediate substrate 120 using a silicone-based adhesive and slightly compressed between the film layer 125 and the intermediate substrate 120. In a variation of the specific 30 embodiment, the elastomeric layer 140 may alternatively be held in place solely by pressure between the intermediate layer 120 and the top layer 110. Preferably, the elastomeric layer 140 is reversibly deformable over the usage lifetime of the microfluidic cartridge 100, such that any occlusion of a 35 microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 is reversible over the usage lifetime of the microfluidic cartridge. Alternatively, the elastomeric layer 140 may not be reversibly deformable, such that an occlusion of a microfluidic channel of a fluidic pathway 165 40 contacting the elastomeric layer 140 is not reversible.

The set of occlusion positions 141 preferably comprises at least two types of occlusion positions, as shown in FIG. 1C, including a normally open position 42 and a normally closed position 43. As shown in FIGS. 5A-5D, the elastomeric layer 45 140 at a normally open position 42 of the set of occlusion positions 141 may be closed upon occlusion by an occluding object (FIGS. 5B and 5D). Preferably, a normally open position 42 is configured to withstand pressures that can be generated by a fluid delivery system (e.g. a syringe pump) with- 50 out leaking, upon occlusion by an occluding object at the normally open position 42. In one specific example, a ½ barrel-shaped pin head may be used to fully occlude a normally open position 42 having an arched cross section, as in FIG. 5C, with near constant pressure on the portion of the 55 elastomeric layer compressed between the occluding object and occluding position.

The normally closed position 43 of the set of occlusion positions 141, functions to be normally closed, but to be forced open in response to fluid delivery by a fluid delivery 60 system. In one variation, the normally closed position 43 may be formed by manufacturing (e.g. injection molding) the top layer 100, such that the top layer material at a normally closed position 43 extends down to the elastomeric layer 140. If an occluding object is held away from the normally closed posi- 65 tion 43, the occlusion position is closed, but can be forced open due to fluid pressure applied by a fluid delivery system

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(e.g. syringe pump). When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.

The microfluidic cartridge 100 may further comprise a bottom layer 170 configured to couple to the intermediate substrate, which functions to allow waste to be contained within the microfluidic cartridge 100, and allow microfluidic cartridges to be stacked. The bottom layer thus facilitates reception, isolation, and containment of a waste fluid within the waste chamber. Preferably, the bottom layer 170 is composed of the same material as the intermediate substrate 120 for cost and manufacturing considerations, and bonded to the intermediate substrate 120 in a manner that provides a hermetic seal, such that a liquid within the waste chamber 130 does not leak out of the waste chamber 130. In a specific embodiment, the bottom layer 170 and the intermediate substrate 120 are both composed of a polypropylene-based material, and bonded together using an adhesive. In an embodiment of the microfluidic cartridge 100 where the waste chamber 130 has a corrugated surface, the bottom layer 170 preferably only seals voids defining the waste chamber 130, such that non-waste chamber regions (i.e. non-waste housing regions) are not covered by the bottom layer 170. Alternatively, the microfluidic cartridge 100 may omit the bottom layer 170, such that any waste fluid that enters the waste chamber 130 completely leaves the microfluidic cartridge 100 and is collected off-cartridge by a waste-collecting subsystem of an external molecular diagnostic system. In this alternative embodiment, the intermediate substrate 120 is configured to fluidically couple to the waste-collecting subsystem.

## 1.4 Microfluidic Cartridge—Magnet Housing

The magnet housing region 150 of the microfluidic cartridge 100 functions to provide access to and/or house at least one magnet 152 providing a magnetic field 156 for purification and isolation of nucleic acids. Preferably, the magnet housing region 150 is defined by the film layer and the intermediate substrate, such that the film layer and the intermediate substrate form the boundaries of the magnet housing region 150. In an embodiment of the microfluidic cartridge 100 comprising a bottom layer 170, the magnet housing region 150 may further be defined by the bottom layer 170, such that the bottom layer partially forms a boundary of the magnet housing region 150. The magnet housing region 150 is preferably a rectangular prism-shaped void in the microfluidic cartridge 150, and accessible only through one side of the microfluidic cartridge 100, as shown in FIG. 1B. Preferably, the magnet housing region 150 can be reversibly passed over a magnet 152 to house the magnet 152, and retracted to remove the magnet 152 from the magnet housing region 150; however, the magnet 152 may alternatively be irreversibly fixed within the magnet housing region 150 once the magnet 152 enters the magnet housing region 150.

Preferably, the magnet housing region 150 is bounded on at least two sides by the waste chamber 130, and positioned near the middle of the microfluidic cartridge 100, such that a fluidic pathway 165 passing through the magnetic field 156 passes through the magnetic field 156 at least at one point along an intermediate portion of the fluidic pathway 165. Preferably, the magnet housing region 150 also substantially spans at least one dimension of the microfluidic cartridge, such that multiple fluidic pathways 165 of the microfluidic

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cartridge 100 cross the same magnet housing region 150, magnet 152, and/or magnetic field 156. Alternatively, the magnet housing region 150 may be configured such that a magnet within the magnet housing region 150 provides a magnetic field spanning all fluidic pathways 165 of the 5 microfluidic cartridge in their entirety. In alternative embodiments, the microfluidic cartridge may comprise more than one magnet housing region 150, a magnet housing region 150 may be configured to receive and/or house more than one magnet 152, and/or may not be positioned near the middle of 10 the microfluidic cartridge 100. In yet another alternative embodiment, the magnet housing region 150 may permanently house a magnet 152, such that microfluidic cartridge comprises a magnet 152, integrated with the intermediate substrate 120. In embodiments where the magnet 152 is 15 retractable from the microfluidic cartridge 100, the magnet 152 may be a permanent magnet or an electromagnet. In embodiments where the magnet 152 is configured to be integrated with the microfluidic cartridge 100, the magnet 152 is preferably a permanent magnet, which provides a stronger 20 magnetic field per unit volume.

#### 1.5 Microfluidic Cartridge—Fluidic Pathways

The set of fluidic pathways 160 of the microfluidic cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used 25 in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a detection chamber for analysis, which may include amplification and/ or detection. Preferably, each fluidic pathway 165 in the set of 30 fluidic pathways 160 is formed by at least a portion of the top layer, a portion of the film layer, and a portion of the elastomeric layer 140, such that each fluidic pathway 165 may be occluded upon deformation of the elastomeric layer 140 at a set of occlusion positions 141. Additionally, at least one flu- 35 idic pathway 165 in the set of fluidic pathways 160 is preferably fluidically coupled to a sample port-reagent port pair 113 of the set of sample port-reagent port pairs 112, a fluid port 118, a waste chamber 130, and a detection chamber 117 of the fluidic pathway 165 in the set of fluidic pathways 160 is preferably configured to be occluded upon deformation of the elastomeric layer 140, configured to transfer a waste fluid to the waste chamber 30, comprises a capture segment 166 passing through the heating region 195 and a magnetic field 45 156, and is configured to pass through the vent region 190 upstream of a detection chamber 117. Alternative embodiments may omit preferred elements of the embodiment of the fluidic pathway 165 described above, such as a vent region embodiment of the fluidic pathway 165 described above.

A fluidic pathway 165 of the set of fluidic pathways 160 may comprise portions (i.e. microfluidic channels) that are located on both sides of the top layer 110, but is preferably located primarily on the bottom side of the top layer (in the 55 orientation shown in FIG. 1B). In the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a microfluidic channel on top of the top layer 110 may be further covered by second film layer 168 that seals the microfluidic channel on top of the top layer 110. The second film layer 168 may be 60 comprise a cyclic olefin polymer (COP) film, thermally or adhesively bonded to the top layer 110, or alternatively may comprise another material that is bonded to the top layer 110. The use of film layers 125, 168 to cover microfluidic channels on either side of the top layer 11 facilitates manufacturing, such that long stretches of a fluidic pathway 165 do not need to be produced within the interior of the top layer 110. Pref-

erably, microfluidic channels may be etched, formed, molded, cut, or otherwise shaped into the rigid structure of the top layer 110, and either remain on one side of the top layer

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110, or pass through the thickness of the top layer 110.

In one variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 11B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a vent region 190 on the top side of the top layer 110. All other segments of the fluidic pathway 165 are preferably located on the bottom side of the top layer 110, allowing the fluidic pathway 165 to be sealed by the film layer 125 without requiring a separate film layer to seal channels located on the top of the top layer 110.

In another variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a detection chamber 163 on the top side of the top layer 110 and a segment running away from the detection chamber 164 on the top side of the top layer 110. In this variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 upstream of the first segment running to the detection chamber 163, and crosses the thickness of the top layer 110 downstream of the segment running away from the detection chamber 164, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. In another variation, as shown in FIG. 6C, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising only a segment running away from the detection chamber 164 on the top side of the top layer 110. In this other variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 downstream of the second portion, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. Alternatively, other embodiments may comprise a fluidic pathway 165 with a different configuration of portions on the top side of the top layer 110 and/or portions on the bottom side of the top layer 110.

As shown in FIGS. 1C, 6C, 7 and 9, a fluidic pathway 165 set of detection chambers 116. Furthermore, at least one 40 of the set of fluidic pathways 160 is branched and preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118, a sample segment 175 coupled to a sample port 114, a reagent segment 176 coupled to a reagent port 115, a capture segment 166 passing through at least one of the heating region 195 and a magnetic field 156, a vent segment 177 configured to pass through the vent region 190, a segment running to a detection chamber 163, a segment running away from the detection chamber 164, and at least one waste segment 178, 179 configured to transfer a waste 190 or a heating region 195, or add additional elements to the 50 fluid to a waste chamber 130. Individual segments of the fluidic pathway 165 are preferably configured to pass through at least one occlusion position of the set of occlusion positions 141, to controllably direct fluid flow through portions of the fluidic pathway 165. A fluidic pathway 165 may also further comprise an end vent 199, which functions to prevent any fluid from escaping the microfluidic channel.

The initial segment 174 of the fluidic pathway 165 functions to deliver common liquids and/or gases from a fluid port 118 through at least a portion of the fluidic pathway 165, the sample segment 175 functions to deliver a volume of a sample fluid (e.g. sample comprising nucleic acids bound to magnetic beads) to a portion of the fluidic pathway 165, and the reagent segment 176 functions to deliver a volume of fluid comprising a reagent to a portion of the fluidic pathway 165. The capture segment 166 functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid, and may be s-shaped and/or progressively narrowing, to

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increase the efficiency and/or effectiveness of isolation and purification. Alternatively, the capture segment 166 may altogether be replaced by a substantially straight portion 166 or any other geometric shape or configuration that functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid. The capture segment 166 of the fluidic pathway 165 preferably has an aspect ratio less than one, which functions to facilitate capture of magnetic particles, but may alternatively have an aspect ratio that is not less than one.

The vent segment 177 functions to deliver a processed sample fluid through the vent region 190 for gas removal. The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment 15 running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117. The segments may be arranged in at least one of several configurations to facilitate isolation, processing, and amplification of a nucleic acid sample, as described in three exemplary 20 embodiments below:

A first embodiment, as shown in FIG. 1C, of a fluidic pathway 165 preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a shared fluid port 118, a sample segment 175 coupled to a sample port 25 114 and to the initial segment 174, and an s-shaped capture segment 166, configured to pass through the heating region 195 and a magnetic field 156, coupled to the initial segment 174 and the sample segment 175. In a variation of the first embodiment, the s-shaped capture segment 166 may com- 30 prise an initial wide arc 166 to provide a greater surface area for magnetic bead capture. In another variation of the first embodiment, the capture segment 166 may alternatively be a progressively narrowing s-shaped capture segment 166. The first embodiment of the fluidic pathway 165 also comprises a 35 reagent segment 176 coupled to a reagent port 115 and to the capture segment 166, a vent segment 177 coupled to the reagent segment 176 and configured to pass through the vent region 190, a segment running to a detection chamber 163 from the vent region 190, a winding segment running away 40 from the detection chamber 164, and an end vent 199 coupled to the segment running away from the detection chamber 164. The first embodiment of the fluidic pathway 165 also comprises a first waste segment 178 configured to couple the initial segment 174 to the waste chamber 130, and a second waste segment 179 configured to couple the capture segment 166 to the waste chamber 130. The first waste segment 178 preferably functions to allow evacuation of excess release fluids from a fluidic pathway 165, for precise metering of the amount of release reagents used in a molecular diagnostic 50 procedure using a low volume of sample.

In the first embodiment, the set of occlusion positions 141 comprises a first occlusion position 142 located along the initial segment 174 between points at which the initial segment couples to the fluid channel 119 and to the capture 55 segment 166. The set of occlusion positions 141 also comprises a second occlusion position 143 located along the sample segment 175, a third occlusion position 144 located along the reagent segment 176, a fourth occlusion position 145 located along the first waste segment 178, and a fifth 60 occlusion position 146 located along the second waste segment 179. In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the 65 segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running

away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145,

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and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.

The occlusion positions of the set of occlusion positions 141 of the first embodiment are preferably located such that occluding subsets of the set of occlusion positions 141 defines unique truncated fluidic pathways to controllably direct fluid flow. For example, as shown in FIG. 1D, occluding the fluidic pathway 165 at the first, third, fourth, and sixth occlusion positions 142, 144, 145, 147 forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port 114, may flow past the second occlusion positions 143 into the capture segment 166 for isolation and purification of nucleic acids using the heating region 195 and the magnetic field 156. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment 166 by the magnetic field 156, while other substances in the volume of sample fluid may pass into the waste chamber 130 by passing the fifth occlusion position 146. Following this subset of occlusion positions, the occlusion at the first occlusion position 142 may be reversed, as shown in FIG. 1E, and the fluidic pathway 165 may be occluded at the second occlusion position 143 to form a second truncated pathway by which a wash fluid may be delivered through the fluid port 118, into the capture segment 166 (thus washing the trapped magnetic beads), and into the waste chamber 130 by passing the fifth occlusion position 146. The occlusion at the second occlusion position 143 may then be reversed, and the first occlusion position 142 may be occluded (as shown in FIG. 1D), so that other fluidic pathways in the set of fluidic pathways 160 may be washed. After all fluidic pathways have been washed, a volume of air may be transferred through the fluid port 118 to prevent mixture of a wash solution with a release solution.

Thereafter in the first embodiment, as shown in FIG. 1E, the fluidic pathway 165 may be occluded at the second occlusion position 143 and the occlusion at the first occlusion 142 may be reversed, thus creating a third truncated pathway as shown in FIG. 1D. A release solution may then be delivered through the fluid port 118, into the capture segment 166, and to the waste chamber 130 by passing the fifth occlusion position 146. The release solution may then be sealed within a fourth truncated pathway (including the capture segment 166) of the fluidic pathway 165 by occluding the fluidic pathway at the fifth occlusion position 146, as shown in FIG. 1F. A release solution may then be delivered to other fluidic pathways of the set of fluidic pathways 160.

Thereafter, as shown in FIG. 1G, the occlusion at the fourth occlusion position 145 may be reversed, creating a fifth truncated pathway, and release solution within the fluidic pathway 165 may be metered by pumping air through the fluid port 118, which functions to push a portion of the release solution into the waste chamber 130. A volume of release solution will still be maintained within the capture segment 166 at this stage. As shown in FIG. 1H, the first and the fourth occlusion positions 142, 145 may then be occluded to form a sixth truncated pathway sealing the volume of release solution, with the captured magnetic beads bound to nucleic acids, within the capture segment 166. The volume of the remaining release solution is therefore substantially defined by the microchannel volume between junctions in the fluidic pathway 165 near the fourth and sixth occlusion positions 145, 147, and may be any small volume but in a specific variation is precisely metered to be 23+/-1 microliters.

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Release solution may be sealed within capture segments of other fluidic pathways using a similar process. A heater may then be provided at the sixth truncated pathway, inducing a pH shift within the sixth truncated pathway to unbind nucleic acids from the magnetic beads.

Thereafter in the first embodiment, as shown in FIG. 1I, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. ~20 microliters) may be aspirated out of the microfluidic cartridge through the 10 reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion position 147 may be reversed, and the fluidic pathway 165 may be occluded at the 15 first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be dispensed through the reagent port 115, through the eighth 20 truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position (normally closed) open. The detection chamber 117 is completely filled with the mixed reagent-nucleic acid sample, after which the fluidic pathway **165** is occluded at the 25 third, sixth, seventh and eighth occlusion positions 144, 147, **148**, **149**, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module 30 may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection chamber 117.

An alternative variation of the first embodiment may further comprise additional occlusion positions or alternative 35 variations of the set of occlusion positions 141, such that occlusion at the additional occlusion positions permanently seals the waste chamber from the fluidic pathway 165. Other alternative variations of the first embodiment may also comprise configurations of the set of occlusion positions 141 that 40 are different than that described above. The variations may be configured, such that the a fluidic pathway 165 facilitates meter release, does not allow meter release, facilitates addition of other reagents (e.g. neutralization or DNase reagents), facilitates additional washing steps, and/or facilitates other 45 operations without changing the layout of the fluidic pathway 165 of a microfluidic cartridge embodiment. Thus, multiple unique operations may be performed using the same microfluidic cartridge, by occluding fluidic pathways 160 at varied subsets of a set of occlusion positions 141.

A second embodiment, as shown in FIG. 6C, of a fluidic pathway 165' preferably comprises an initial segment 174' fluidically coupled to a fluid channel 119' coupled to a shared fluid port 118', a sample segment 175' coupled to a sample port 114' and to the initial segment 174', and a capture seg- 55 ment 166', configured to pass through the heating region 195 and a magnetic field 156, coupled to the initial segment 174'. The second embodiment of the fluidic pathway 165' also comprises a reagent segment 176' coupled to a reagent port 115' and to the turnabout portion 176', a vent segment 177' 60 coupled to the reagent segment 176' and to the capture segment 166' and configured to pass through the vent region 190, a segment running to a detection chamber 163' from the vent region 190, a segment running away from the detection chamber 164', and an end vent 199 coupled to the segment running away from the detection chamber 164'. The second embodiment of the fluidic pathway 165' also comprises a first waste

segment 178', coupled to the initial segment 174' at a point between points connecting the initial segment 174' to the sample segment 175' and to the capture segment 166'. The first waste segment 178' is configured to couple the initial segment 174' to the waste chamber 130. The second embodiment of the fluidic pathway 165' also comprises a second waste segment 179' configured to couple the capture segment 166' to the waste chamber 130', and an end vent segment 197' coupled to the capture segment 166' downstream of the point of connection to the second waste segment 179', and coupled to an end vent 199. The end vent segment 197' functions to

provide fine metering of a fluid flowing through the fluidic

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pathway 165'. In the second embodiment, the set of occlusion positions 141' comprises a first occlusion position 142' located along the initial segment 174' between points at which the initial segment couples to the fluid channel 119' and to the sample segment 175'. The set of occlusion positions 141' also comprises a second occlusion position 143' located along the sample segment 175', a third occlusion position 144' located along the reagent segment 176', a fourth occlusion position 145' located along the first waste segment 178', and a fifth occlusion position 146' located along the second waste segment 179'. In the second embodiment, the set of occlusion positions 141' also comprises a sixth occlusion position 147' located along the vent segment 177' upstream of the vent region 190, a seventh occlusion position 148' located along the segment running to the detection chamber 163', and an eighth occlusion position 149' located along the segment running away from the detection chamber 164'. Additionally, in the second embodiment, the set of occlusion positions 141 comprises a ninth occlusion position 157' located along the sample segment 175' between the sample port 114 and the second occlusion position 143, a tenth occlusion position 158' located along the end vent segment 197, and an eleventh occlusion position 159' located along the capture segment 166' between points at which the capture segment 166' couples to the end vent segment 197' and to the vent segment

The occlusion positions of the set of occlusion positions **141'** of the second embodiment are preferably located such that occluding of subsets of the set of occlusion positions 141' defines unique truncated fluidic pathways to controllably direct fluid flow. For example, occluding the fluidic pathway 165' at the first, fourth, sixth, tenth, and eleventh occlusion positions 142', 145', 147', 158', 159' forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port 114, may flow into the capture segment 166' for isolation and purification of nucleic acids using the heating region 195 and the magnetic field 156. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment 166' by the magnetic field 156, while other substances in the volume of sample fluid may pass into the waste chamber 130 by passing the fifth occlusion position 146'. Following this subset of occlusion positions, the occlusion at the first occlusion position 142' may be reversed, and the fluidic pathway 165' may be occluded at the second occlusion position 143' to form a second truncated pathway by which a wash fluid may be delivered through the fluid port 118, into the capture segment 166' (thus washing the trapped magnetic beads), and into the waste chamber 130 by passing the fifth occlusion position 146'. A volume of air may then be pumped through the fluid port 118 to flush any remaining wash solution into the waste chamber 130.

Thereafter, in the second embodiment, the fluidic pathway 165' may be occluded at the fifth occlusion position 146' and

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the occlusion at the tenth occlusion position 158' may be reversed, closing access to the waste chamber 130 and opening access to the end vent segment 197'. A release solution may then be delivered through the fluid port 118, into the capture segment 166', and to the end vent segment 197'. The 5 volume of the release solution is therefore defined by the microchannel volume between the fourth and tenth occlusion positions 145', 158', and may be any small volume but in a specific variation is precisely metered to be 15 microliters. Thereafter, occluding the fluidic pathway 165' at the tenth 10 occlusion position 158', reversing the occlusion at the fourth occlusion position 145' (defining a fourth truncated pathway), and delivering air through the fluid port 118 pushes any remaining release buffer from the fluidic pathway 118 into the waste chamber 130, thereby ensuring that excess release 15 buffer is not later exposed to nucleic acids bound to the magnetic beads (at this point, the nucleic acids are not substantially released from the magnetic beads because heat has not been added). Thereafter, the fluidic pathway 165' is occluded at the first and fourth occlusion positions 142', 145', 20 defining a fifth truncated pathway comprising the capture segment 166', and the magnetic beads are heated to an appropriate temperature and time (e.g., 60 degrees for 5 minutes) within the heating region 195 to release the nucleic acids from the magnetic beads and into the release buffer.

Thereafter, in the second embodiment, the occlusions at the first and eleventh occlusion positions 142', 159' are reversed, defining a sixth truncated pathway, the entire released nucleic acid sample (e.g. ~15 microliters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This 30 released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent mixture stored off of the microfluidic cartridge 100. During the reconstitution process, the occlusion at the sixth occlusion position 147' may be reversed, thus defining a seventh truncated pathway. Once 35 reconstitution of the molecular diagnostic reagent mixture with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be aspirated through the reagent port 115 through the seventh truncated pathway to the detection chamber 117, completely filling the 40 detection chamber 117, after which the fluidic pathway 165' is be occluded at third, seventh, eighth, and ninth occlusion positions 144', 148', 149', 157' defining an eighth truncated pathway. An external molecular diagnostic system and/or module may then perform additional processes on the volume 45 of fluid within the detection chamber 117.

An alternative variation of the second embodiment may further comprise additional occlusion positions or alternative variations of the set of occlusion positions 141', such that occlusion at the additional occlusion positions permanently 50 seals the waste chamber from the fluidic pathway 165'. Other alternative variations of the second embodiment may also comprise configurations of the set of occlusion positions 141' that are different than that described above.

A third embodiment, as shown in FIG. 7, of a fluidic pathway 165" preferably comprises an initial segment 174" fluidically coupled to a fluid channel 119" coupled to a shared fluid port 118, a sample segment 175" coupled to a sample port 114 and to the initial segment 174", and a capture segment 166" coupled to the initial segment 174". The third embodiment of the fluidic pathway 165" also comprises a reagent segment 176" coupled to a reagent port 115, a vent segment 177" coupled to the reagent segment 176" and to the capture segment 166", and configured to pass through the vent region 190, a segment running to a detection chamber 65 163" from the vent region 190, a segment running away from the detection chamber 164", and an end vent 199 coupled to

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the segment running away from the detection chamber 164". The third embodiment of the fluidic pathway 165" also comprises a first waste segment 178" configured to couple the initial segment 174" to the waste chamber 130, and a second waste segment 179" configured to couple the capture segment 166" to the waste chamber 130.

In the third embodiment, the set of occlusion positions 141" comprises a first occlusion position 142" located along the initial segment 174" between points at which the initial segment 174" couples to the fluid channel 119" and to the sample segment 175". The set of occlusion positions 141" also comprises a second occlusion position 143" located along the sample segment 175", a third occlusion position 144" located along the reagent segment 176", a fourth occlusion position 145" located along the first waste segment 178", and a fifth occlusion position 146" located along the second waste segment 179". In the third embodiment, the set of occlusion positions 141" also comprises a sixth occlusion position 147" located along the vent segment 177" upstream of the vent region 190, a seventh occlusion position 148" located along the segment running to the detection chamber 163", an eighth occlusion position 149" located along the segment running away from the detection chamber 164", and a ninth occlusion position 157" located along the vent seg-25 ment 177" between the point at which the vent segment 177" couples to the second waste segment 179" and the sixth occlusion point 147".

Similar to the first and the second embodiments, the occlusion positions of the set of occlusion positions 141" of the third embodiment are preferably located such that an occlusion of subsets of the set of occlusion positions 141" defines unique truncated fluidic pathways to controllably direct fluid flow. Example truncated fluidic pathways, defined by occluding the fluidic pathway 165" using subsets of the set of occlusion positions 141", are shown in FIG. 7.

Preferably, a fluidic pathway 165 of the set of fluidic pathways 160 comprises at least one of a first channel type 171, a second channel type 172 with a reduced cross sectional area, and a third channel type 173 with an curved surface as shown in FIG. 8A. A variation of the first channel type 171 has an approximately rectangular cross section with slightly sloping walls, such that at least two walls of the first channel type 171 slope toward each other to facilitate manufacturing of the first channel type 171; however, alternative variations of the first channel type 171 may have non-sloping walls or walls that slope away from each other. In specific embodiments of the first channel type 171, the walls of the first channel type 171 slope at 6° from vertical, to facilitate extraction of injection molded parts, and are between 300 and 1600 microns wide and between 100 and 475 microns tall. In a first specific embodiment of the second channel type 172, the cross section of the second channel type 172 is a 250 micron wide equilateral triangle with the top truncated to be 200 microns deep. In a second specific embodiment of the second channel type 172, the cross section of the second channel type is a truncated triangle that is 160 microns wide and 160 microns deep. In a specific embodiment of the third channel type 173, the surface of the third channel type is defined by Gaussian function, and is 800 microns wide and 320 microns deep. Alternative embodiments of the third channel type 173 may comprise a surface defined by any appropriate curved function.

The first channel type 171 is preferably used over a majority of a fluidic pathway 165, and preferably in portions near a vent region 190, in a capture segment 166 configured to pass through a magnetic field 156, and in a segment leading to a Detection chamber 163. Preferably, an embodiment of the first channel type 171, comprising a wide channel with little

ate element.

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depth is used in regions configured to pass through a magnetic field 156, such that particles in the regions are driven closer to the magnetic field source. The second channel type 172 is preferably used near a vent region 190 of a fluidic pathway 165, and preferably in portions of a fluidic pathway 165 5 leading to and away from a detection chamber 163, 164 (to constrict fluid flow into the Detection chamber 117). The third channel type 173 is preferably used in a portion of a fluidic pathway 165 near a normally open position 42 of the set of occlusion positions 141. Transitions between different channel types 171, 172, 173 may be abrupt, or alternatively, may be gradual, as shown in FIG. 8B. The first, second, and third channel types 171, 172, 173 may also alternatively be used in any appropriate portion of a fluidic pathway 165. Example embodiments of channel types for segments of a fluidic path- 15 way are shown in FIG. 8C.

Multiple fluidic pathways may be configured to pass through a single heating region 195 of the microfluidic cartridge 100, a single vent region 190 of the microfluidic cartridge 100, and/or a magnetic field 156 produced by a magnet 20 152 housed within a single magnet housing region 150. Preferably all fluidic pathways of the set of fluidic pathways 160 are configured to pass through a single heating region 195 of the microfluidic cartridge 100, and a magnetic field 156 produced 25 by a magnet 152 housed within a single magnet housing region 150; however, alternative embodiments of the set of fluidic pathways 160 of the microfluidic cartridge may comprise different configurations wherein fluidic pathways of the set of fluidic pathways 160 do not share a single heating 30 region 195, a single vent region 190, and/or a magnetic field 156

Additionally, the set of fluidic pathways 160 of the microfluidic cartridge 100 may comprise virtually any number of fluidic pathway 165 and/or the set of Detection chambers 116 may comprise virtually any number of Detection chambers 116 as can practically be integrated into the microfluidic cartridge 100. In one specific embodiment, the set of fluidic pathways 160 may comprise twelve fluidic pathways 165, four of which are shown in FIG. 9.

1.6 Microfluidic Cartridge—Additional Microfluidic Cartridge Elements

The microfluidic cartridge 100 is preferably configured such that actual valving members are not integrated into the microfluidic cartridge 100, thus, opening and/or occluding 45 portions of a fluidic pathway 165 are performed by systems located external to the microfluidic cartridge. As an example, portions of a fluidic pathway 165 may be opened or occluded at occlusion positions, as described above, by the action of a valving member or mechanism held beneath the card that 50 applies a biasing force to deform the elastomeric layer 140 and occlude a fluidic pathway 165. The force may be applied by a mechanical member (e.g., a pin, post, etc.), an electromechanical member (e.g. a solenoid), a pneumatic or hydraulic member (e.g., air, water, etc.) or any other appropriate 55 means, as shown in FIGS. 10A and 10B. In some variations, the cartridge may include one or more registration regions that allow the card to be aligned with respect to the valving member or mechanism. In alternative embodiments, the elastomeric layer 140, the set of valve guides 127, and the set of 60 occlusion positions 141 may be omitted and replaced with valves integrated within the microfluidic cartridge 100, that are configured to controllably occlude and open portions of a fluidic pathway 165.

Other embodiments of the microfluidic cartridge 100 may 65 further comprise a tag 198 that functions to encode and provide identifying information related to the microfluidic car-

tridge 100. The tag 198 may comprise a barcode, QR code, or other optical machine-readable tag, or may alternatively be an electronic tag, such as an RFID chip. The identifying information preferably comprises at least information relating to the position of a microfluidic cartridge 100 within a molecular diagnostic system, and information relating to samples analyzed using the microfluidic cartridge 100 (e.g. how many positions remain available for conducting tests). In alternative variations, the tag may relate other information about samples (e.g. sample type, sample volume, sample concentration, date) processed using the microfluidic cartridge 100. Preferably, the tag does not interfere with procedures being performed using the microfluidic cartridge, and is located in an unobtrusive position on the microfluidic cartridge 100, such as a side panel of the microfluidic cartridge 100. Alternatively, the microfluidic cartridge 100 may not comprise a tag 198, and a user or other entity may relate identifying information to the microfluidic cartridge 100 using any appropri-

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As a person skilled in the art will recognize from the previous detailed description and from the FIGURES and claims, modifications and changes can be made to the preferred embodiments of the microfluidic cartridge 100 without departing from the scope of this invention, as is shown in the example embodiment shown in FIGS. 11A and 11B, and in the alternative example embodiment of FIGS. 6A-6C, wherein in the orientation of FIG. 6B, the intermediate substrate 120 comprising a waste chamber 130 is coupled to the top layer 110, and the elastomeric layer 140 is located on the bottom of the microfluidic cartridge 100.

2. Specific Embodiment of a Microfluidic Cartridge

The following description a specific embodiment of the microfluidic cartridge 100 is for illustrative purposes only, and should not be construed as definitive or limiting of the scope of the claimed invention.

The specific embodiment of the microfluidic cartridge 100, as shown in FIGS. 11A and 11B, meets SLAS ANSI guidelines for a microtiter plate footprint, governing the dimensions of the specific embodiment of the microfluidic cartridge 100. The specific embodiment of the microfluidic cartridge 100 is thus 127.76 mm long and 85.48 mm wide.

The specific embodiment of the microfluidic cartridge 100 comprises a top layer 110 including a set of twelve sample port-reagent port pairs 112, a set of twelve Detection chambers 116, a shared fluid port 118, a heating region 195, and a vent region 190, an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer 110 by a film layer 125, configured to form a waste chamber 130, an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber, and a set of fluidic pathways 160, formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140.

The top layer 110 of the specific embodiment of the microfluidic cartridge 100 functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR. The majority of the top layer 110 of the specific embodiment is 1.5 mm thick (aside from regions defining ports, the vent, the heating region 195 or fluidic pathways 165), and is produced by injection molding without the use of a mold release. The polypropylene is clear to allow transmission of light in the detection chambers. The injection molding process defines the set of 12 sample port-reagent port pairs, which are located along one long edge of the top layer 110,

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and also defines the set of 12 detection chambers 116, which are located along the opposite long edge of the top layer 110. The Detection chambers 117 do not completely transect the top layer 110, as shown in FIGS. 11A and 11B. Each detection chamber 117 of the specific embodiment is identical and 5 comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total volume of ~10 mL for each detection chamber 117. The dimensions of the detection chambers 117 of the specific embodiment are such that the detection chambers 117 facilitate heating from one side (resulting in simpler heater design yet fast cycling given the small depth of the channels), and also facilitate the injection molding process. The bottoms of the detection chambers 117 are formed by the film layer 125, which is polypropylene film compatible with PCR (100 microns thick or less) that offers low autofluorescence. The film layer 125 can withstand temperatures up to 120° C. or more.

The injection molding process also defines the shared fluid 20 port 118 of the top layer 110, and the vent region 190, which is recessed 0.5 mm into the top surface of the top layer 110 (in the orientation shown in FIG. 11B), and is covered with a polytetrafluoroethylene membrane, which is hydrophobic, gas permeable, and liquid impermeable. A paper label is 25 bonded with adhesive to the top layer 110 over the vent region 190, which serves to identify the cartridge and protect the vent region 190, as shown in FIGS. 11A and 11B. The injection molding process also defines the heating region 195, which is recessed and spans the long dimension of the top layer 110, 30 slightly offset from a midline of the top layer 110. The top layer 110 of the specific embodiment requires approximately 15 grams of polypropylene, and all draft angles for the top layer 110 are a minimum of 4 degrees, as defined by the injection molding process.

In the specific embodiment, the intermediate substrate 120 is composed of a polypropylene material to minimize cost and simplify assembly, and in the orientation shown in FIG. 11B, the top of the intermediate substrate 120 is 1.5 mm thick. The 120 from the top layer 110 is a polypropylene film with a nominal thickness of 50 microns. The film layer 125 is able to withstand temperatures of up to 95° C. encountered during fabrication and during an intended PCR procedure, while being thermally bondable to the top layer 110. The top layer 45 110 and the film layer 125 are bonded using thermal fusion bonding, and this subassembly is bonded to the intermediate substrate 120 using a polymer adhesive. Additionally, for aligning layers 110, 120, 125 and bonding the top layer 110 to the intermediate substrate 120, plastic studs are configured to 50 extend from the top of the intermediate substrate 120 through die-cut holes in the film layer 125 and injection molded holes in the bottom of the top layer 110. The intermediate substrate also comprises a set of valve guides 127, at a set of occlusion positions 141, which are holes with chamfered edges through 55 the intermediate substrate 127. Each valve guide in the set of valve guides 127 is 2.1 mm×2.1 mm square, and configured to accommodate an occluder with a 2 mm×2 mm square head for normally open positions 42 or 2.1 mm diameter circle to accommodate a 2 mm diameter round pin for normally closed 60 positions 43.

The elastomeric layer 140 of the specific embodiment is composed of a low durometer silicone, and comprises strips that are 500 microns thick and that can withstand temperatures of 120° C. at a minimum. The strips of the elastomeric 65 layer are arranged over the set of valve guides 127, and bonded to the top of the intermediate substrate 120 using a

silicone adhesive. Additionally, the elastomeric layer 140 is slightly compressed between the film layer 125 and the top of the intermediate substrate (in the orientation shown in FIG. 11B).

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The bottom layer 170 of the specific embodiment of the microfluidic cartridge 100 is composed of polypropylene, identical to that of the intermediate substrate 120. The bottom layer is 1.5 mm thick, and is contiguous in the area of the set of Detection chambers 116, such that an outer perimeter of the entire bottom layer 170 substantially spans the footprint of the microfluidic cartridge 100. The bottom layer 170 of the specific embodiment is bonded to the intermediate substrate 120 using polymer adhesive, providing a hermetic seal that ensures that a waste fluid within the waste chamber 130 of the intermediate substrate 120 does not leak out of the waste chamber 130.

The specific embodiment of the microfluidic cartridge 100 comprises twelve fluidic pathways 165 in the set of fluidic pathways 160, such that the microfluidic cartridge 100 is capable of testing up to twelve samples using twelve distinct fluidic pathways 165. Each of the twelve fluidic pathways 165 is coupled to one of the twelve sample port-reagent port pairs 113 on one end of the microfluidic cartridge 100, and coupled to one of the twelve detection chambers 117 on the other end of the microfluidic cartridge, as shown in FIGS. 11A and 11B. Each fluidic pathway 165 is substantially identical (aside from portions connecting to an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118) and identical to the first embodiment of a fluidic pathway described in Section 1.5 and shown in FIG. 1C. Additionally, the microfluidic channels comprising each fluidic pathway 165 are of the first channel type 171 and 500 microns wide by 475 microns deep, aside from the microfluidic channels of the segments leading to and away from the detection chambers 35 163, 164, the turnabout portions 166, and the vent segments 177. Also, parallel microfluidic channels of the fluidic pathways 165 of the specific embodiment are typically evenly spaced at 2.25 mm (center-to-center).

The fluidic pathways 165 of the specific embodiment are, film layer 125, partially separating the intermediate substrate 40 in their default condition, open at all occlusion positions, aside from the fourth, seventh, and eighth, occlusion positions 145, 148, 149, as shown in FIG. 1C. Furthermore, the s-shaped capture segment 166 of a fluidic pathway of the specific embodiment is configured to have a volume capacity of 22 μL, have a width of 5.5 mm, and weave back and forth over a magnetic field 156, by crossing the magnet housing region 150. The depth of the s-shaped capture segment 166 is 0.4 mm for the 1.6 mm wide channels and 0.475 for the 0.5 mm narrower channel.

> The specific embodiment also comprises a barcode tag 198 located on a vertical edge of the microfluidic cartridge 100, as shown in FIG. 11A. Additional features of the specific embodiment of the microfluidic cartridge 100 are shown in FIGS. 11A and 11B.

> 3. Assembly Method for an Embodiment of the Microfluidic An embodiment of an assembly method 200 for an embodiment of the microfluidic cartridge 100 is shown in FIGS. 12A-12G. The assembly method 200 preferably comprises aligning the top layer to the film layer and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge S210, compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate S220, bonding the intermediate substrate to the bottom layer S230, installing the vents of the vent region S250, and applying labels and packaging S260.

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Step S210 recites aligning the top layer to the film layer and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge, and functions to create a first subassembly comprising the top layer, the film layer, the elasto- 5 meric layer, and the intermediate substrate. Preferably, the elastomeric layer is glued with silicone to the intermediate substrate; however, the elastomeric layer may alternatively be solely compressed between the top layer/film layer and the intermediate substrate, without any adhesive. Preferably, a 10 first jig is used to align the top layer and the film layer using pins in the jig and holes in the layers, and in an example embodiment of S210, the top layer is first placed face down in the first jig, and the film layer is placed onto the top layer in preparation for thermal bonding using a lamination machine 15 or hot press. In the example embodiment of S210, the elastomeric layer is then fit over ultrasonic welding tabs in of the top layer, as shown in FIGS. 12D and 12F, however, processes other than ultrasonic welding may be used. An adhesive may also be applied around the border of the elastomeric layer, to 20 prevent leakage between the elastomeric layer and the intermediate substrate. Protrusions molded into the top of the intermediate substrate are then passed through alignment holes in the top layer, thus aligning the top layer, the elastomeric layer, and the intermediate substrate of the microfluidic 25 cartridge. In alternative embodiments of S210, any appropriate alignment mechanism may be used to align the top layer, the elastomeric layer, and the intermediate substrate, using for example, a combination of adhesives, frames, and alignment pins/recesses.

Step S220 recites compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate, and functions to seal the layers in order to prevent leakage between the layers. Preferably, S220 forms 35 hermetic seals between the top layer and the elastomeric layer, and the elastomeric layer and the intermediate substrate, in embodiments of S210 where an adhesive application is involved. In an example embodiment of S220, the first jig with the top layer, the elastomeric layer, and the intermediate 40 substrate is placed within an ultrasonic welder to be compressed and ultrasonically welded.

Step S230 recites bonding the intermediate substrate to the bottom layer S230, which functions to form a second subassembly comprising the top layer, the elastomeric layer, the 45 intermediate substrate, and the bottom layer. Preferably, the bottom layer self-aligns with the intermediate substrate as a result of the bottom layer fitting completely inside a recessed flange on the lower portion of the intermediate layer. The bottom layer is preferably thermally bonded to the intermediate layer. Alternatively, the bottom layer may be bonded to the intermediate layer using adhesive or ultrasonic welding, as shown in FIG. 12G.

Step S250 recites installing the vents of the vent region vent region. Step S250 is preferably performed by heat staking the vents in place, but may alternatively be performed using adhesive or solvent bonding process. Following step S250, the assembly method 200 may further comprise certain quality control measures, including pressure testing the 60 microfluidic cartridge S252 by blocking all sample and reagent ports, and injecting air into the fluid port, and removing the finished microfluidic cartridge from the second jig S254. Step S260 recites applying labels and packaging, and functions to prepare the microfluidic cartridge with identifying information using at least a barcode label, and preparing the microfluidic cartridge for commercial sale.

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An alternative embodiment of an assembly method 300, as shown in FIG. 13, comprises thermally bonding the film layer to the top layer to form a first subassembly S310; adding a vent to the first subassembly and applying a label to create a second subassembly S320; applying an adhesive inside a bottom flange of the intermediate substrate and bonding the bottom layer to the intermediate substrate S330; applying a tag to the intermediate substrate to create a third subassembly S340; positioning the elastomeric layer on the third subassembly to create a fourth subassembly S350; applying adhesive to the fourth subassembly S360; and coupling the second subassembly to the fourth subassembly S370.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of methods according to preferred embodiments, example configurations, and variations thereof. It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose systems that perform the specified functions or acts.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

We claim:

- 1. A cartridge for processing a sample, the cartridge comprising:
  - a first layer comprising a sample port and a fluid port at a broad surface of the first layer;
  - an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, and configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a void external to the waste chamber; and
  - a fluidic pathway, superior to the intermediate substrate and at least partially separated from the corrugated surface of the waste chamber by an elastomeric layer, wherein the fluidic pathway is fluidly coupled to the sample port and the fluid port, and wherein, upon deformation of the elastomeric layer at an occlusion position of the fluidic pathway, by way of the void of the corrugated surface, the fluidic pathway is configured to transfer waste fluid through an opening of the intermediate substrate and into an interior portion of the corrugated surface of the waste chamber.
- 2. The cartridge of claim 1, wherein the first layer is a S250, which functions to permanently form the vents of the 55 unitary construction comprising a reagent port, the fluid port, and a detection chamber, and wherein the fluidic pathway is coupled to the reagent port, the fluid port, and the detection chamber.
  - 3. The cartridge of claim 1, wherein the waste chamber comprises at least one waste inlet in communication with the opening of the intermediate substrate, and wherein the corrugated surface of the waste chamber further defines a rectangular prismatic void spanning a long dimension of the cartridge that defines a magnet housing region configured to reversibly receive a magnet from the direction perpendicular to a broad face of the first layer, and configured to cross the fluidic pathway.

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- **4.** The cartridge of claim **3**, wherein the fluidic pathway comprises a capture segment, downstream of the sample port and configured to cross the magnet housing region multiple times
- 5. The cartridge of claim 4, wherein the fluidic pathway is configured to transfer waste fluid of the sample to the waste chamber through a first waste inlet upstream of the capture segment by way of a first waste segment fluidly coupled to the first waste inlet and an initiating portion of the capture segment, and wherein the fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet downstream of the capture segment by way of a second waste segment fluidly coupled to the second waste inlet and a terminating portion of the capture segment.
- **6**. The cartridge of claim **1**, wherein the elastomeric layer is situated between the fluidic pathway and the intermediate substrate, wherein the intermediate substrate provides access to a set of occlusion positions of the fluidic pathway through at least at a subset of a set of voids of the corrugated surface of the waste chamber, such that the fluidic pathway is configured to be occluded through the elastomeric layer at the set of occlusion positions from a direction perpendicular to the broad surface of the first layer.
- 7. The cartridge of claim **6**, wherein at least one void of the set of voids defined by the corrugated surface of the waste chamber is defined by five surfaces of the corrugated surface, including a first pair of parallel surfaces and a second pair of parallel surfaces orthogonal to the first pair of parallel surfaces and orthogonal to the broad surface of the first layer, and a surface, coupled to the first pair of surfaces and the second pair of surfaces and proximal to the first layer, having a set of openings for occlusion of the fluidic pathway at the set of occlusion positions, and wherein occluding the fluidic pathway at a first subset of the set of occlusion positions defines a truncated pathway configured to facilitate transfer of waste <sup>35</sup> fluid of the sample to the waste chamber.
- **8**. A cartridge for processing a sample, the cartridge comprising:
  - a first layer and an intermediate substrate coupled to the first layer and partially separated from the first layer by a film layer, wherein the intermediate substrate is configured to form a sealed waste chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of parallel voids external to the waste chamber; and
  - a first fluidic pathway, formed by at least a portion of the first layer; and
  - a second fluidic pathway in parallel with the first fluidic pathway and formed by at least a portion of the second fluidic pathway, wherein the first fluidic pathway and the second fluidic pathway are each at least partially separated from the corrugated surface by an elastomeric layer, and each fluidic pathway is configured to transfer waste fluid of the sample into the waste chamber through a set of openings of the intermediate substrate.
- 9. The cartridge of claim 8, wherein the elastomeric layer is inferior to the film layer.
- 10. The cartridge of claim 8, wherein the first layer is a unitary construction comprising a first sample port-reagent port pair including a first sample port, a second sample port-reagent port pair including a second sample port, a fluid port, a first detection chamber, and a second detection chamber, wherein the first fluidic pathway is coupled to the first sample port-reagent port pair and the first detection chamber, wherein the second fluidic pathway is coupled to the second sample port-reagent port pair and the second detection chamber.

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ber, and wherein at least one of the first fluidic pathway and the second fluidic pathway is coupled to the fluid port.

- 11. The cartridge of claim 10, further comprising 1) a heating region defined as a recessed region of the first layer that is parallel to the set of voids of the corrugated surface, and 2) a vent region, such that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber.
- 12. The cartridge of claim 10, wherein the fluid port is fluidly coupled to the first fluidic pathway and to the second fluidic pathway.
- 13. The cartridge of claim 9, wherein the first detection chamber comprises a first serpentine-shaped fluidic channel, and the second detection chamber comprises a second serpentine-shaped fluidic channel.
- 14. The cartridge of claim 13, wherein the first serpentineshaped fluidic channel comprises three wide channels directly interconnected by two narrow channels, wherein the three wide channels include a first wide channel comprising a detection chamber inlet into the first detection chamber and a second wide channel comprising a detection chamber outlet from the first detection chamber.
- 15. The cartridge of claim 8, wherein at least one void of the set of voids defined by the corrugated surface of the waste chamber is a rectangular prismatic void spanning a long dimension of the cartridge and defining a magnet housing region configured to cross the first fluidic pathway and the second fluidic pathway.
- 16. The cartridge of claim 8, wherein the elastomeric layer is situated between the first layer and the intermediate substrate, wherein the intermediate substrate provides access to a set of occlusion positions of the first fluidic pathway and of the second fluidic pathway by way of the set of voids of the corrugated surface of the waste chamber, such that the first fluidic pathway and the second fluidic pathway are configured to be occluded upon deformation of the elastomeric layer at subsets of the set of occlusion positions, and wherein the waste chamber is located inferior to the elastomeric layer.
- 17. The cartridge of claim 16, wherein the first fluidic pathway is configured to transfer a first waste fluid of the sample to the waste chamber through a first waste inlet, upon occlusion of a first subset of the set of occlusion positions, by way of a first waste segment fluidly coupled to the first waste inlet and a first portion of the first fluidic pathway proximal the first sample port, and wherein the first fluidic pathway is configured to transfer a second waste fluid of the sample to the waste chamber through a second waste inlet, upon occlusion of a second subset of the set of occlusion positions, by way of a second waste segment fluidly coupled to the second waste inlet and a second portion of the first fluidic pathway substantially downstream of the first sample port.
- 18. The cartridge of claim 16, wherein the first fluidic pathway is configured to transfer a first waste fluid to the waste chamber through a first waste inlet, upon occlusion of a first subset of the set of occlusion positions, by way of a first waste segment fluidly coupled to the first waste inlet and inline with the first fluidic pathway, and wherein the second fluidic pathway is configured to transfer a second waste fluid to the waste chamber through a second waste inlet, upon occlusion of a second subset of the set of occlusion positions, by way of a second waste segment fluidly coupled to the second waste inlet and inline with the second fluidic pathway.

\* \* \* \* \*

# EXHIBIT 28

## (12) United States Patent

#### Brahmasandra et al.

## (10) Patent No.: US 9,499,896 B2

## (45) **Date of Patent:** \*Nov. 22, 2016

#### (54) THERMOCYCLING SYSTEM, COMPOSITION, AND MICROFABRICATION METHOD

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(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35 U.S.C. 154(b) by 116 days.

This patent is subject to a terminal disclaimer.

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(22) Filed: Sep. 16, 2014

(65) **Prior Publication Data** 

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(51) Int. Cl.

 B01L 3/00
 (2006.01)

 B01L 7/00
 (2006.01)

 C23C 14/02
 (2006.01)

(52) U.S. Cl.

(58) Field of Classification Search

CPC .............. B01L 7/525; B01L 2300/1822; B01L 2300/1827; B01L 3/502723; B01L 7/52 See application file for complete search history.

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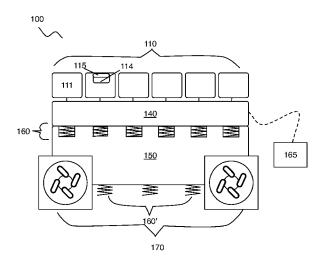
\* cited by examiner

Primary Examiner — Nathan Bowers (74) Attorney, Agent, or Firm — Jeffrey Schox; Ivan Wong

## (57) ABSTRACT

A system and method of manufacture for the system, comprising a set of heater-sensor dies, each heater-sensor die comprising an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region, wherein the pattern of voids in heating region defines a coarse pattern associated with a heating element of the heating region and a fine pattern, integrated into the coarse pattern and associated with a sensing element of the heating region; an electronics substrate configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of elastic elements configured to bias each of the set of heater-sensor dies against a detection chamber.

## 9 Claims, 9 Drawing Sheets



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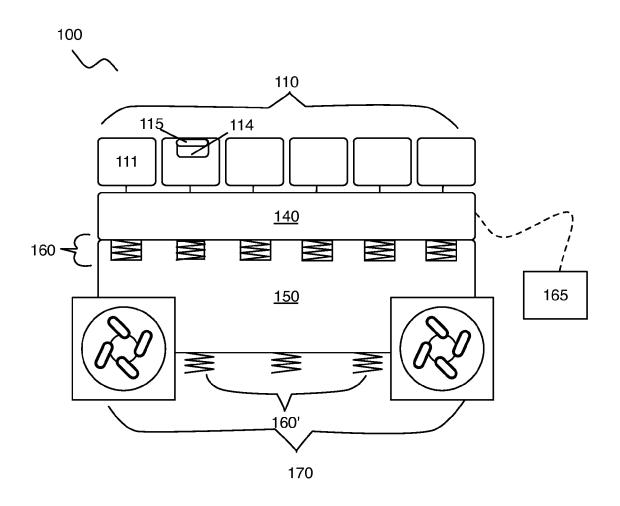
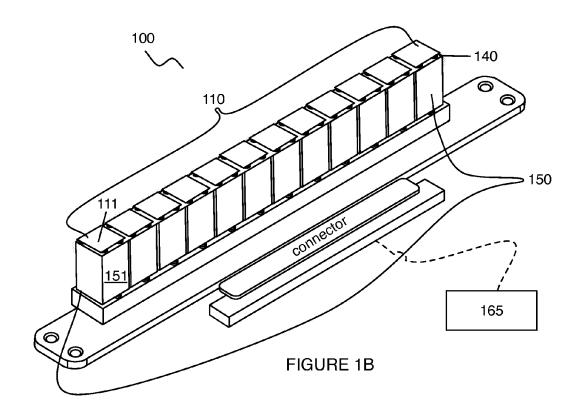
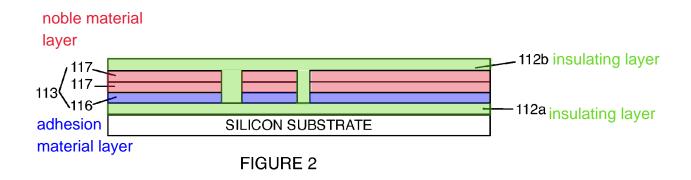


FIGURE 1A

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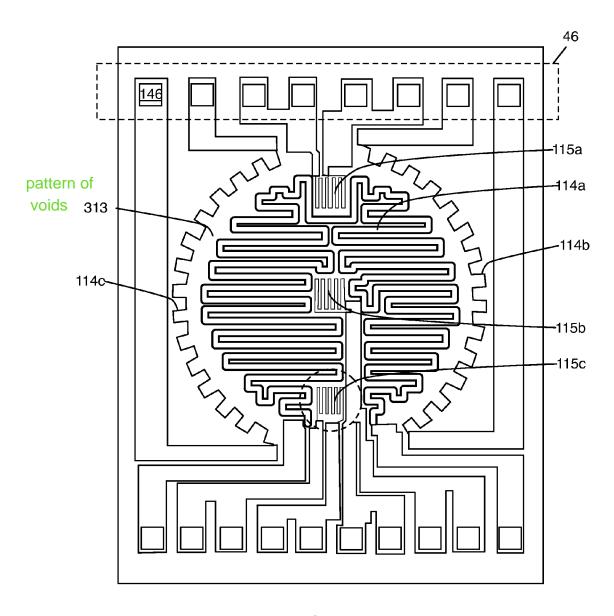


FIGURE 3

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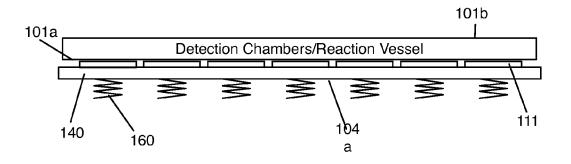


FIGURE 4A

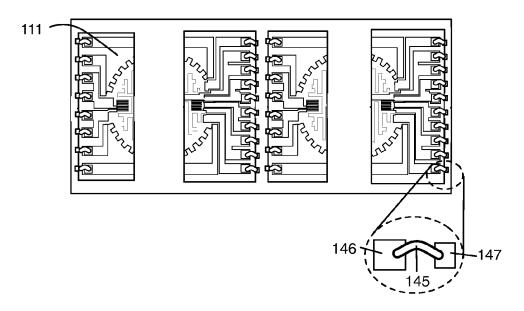


FIGURE 4B

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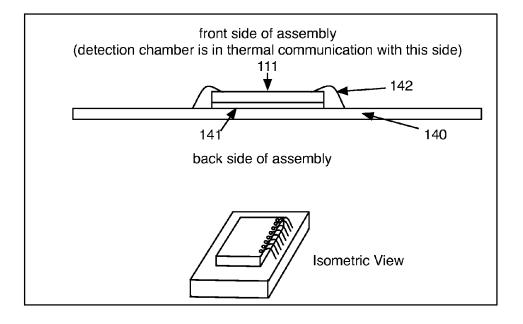


FIGURE 5A

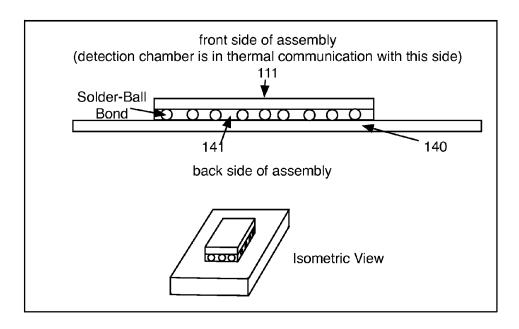


FIGURE 5B

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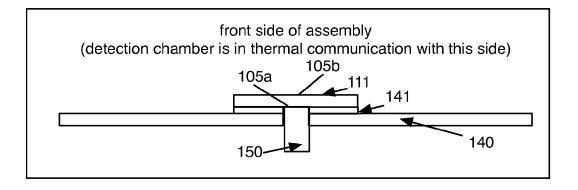


FIGURE 6A

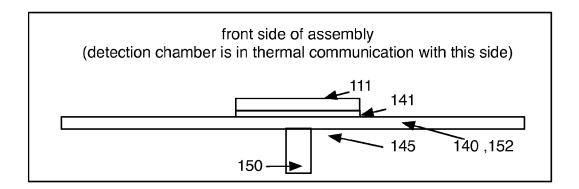
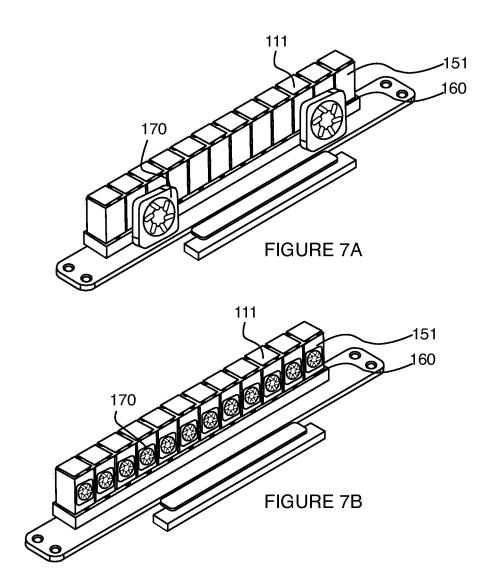


FIGURE 6B

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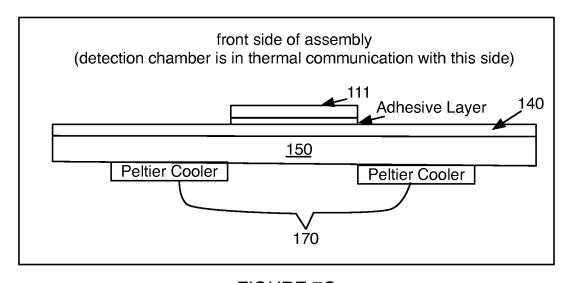


FIGURE 7C

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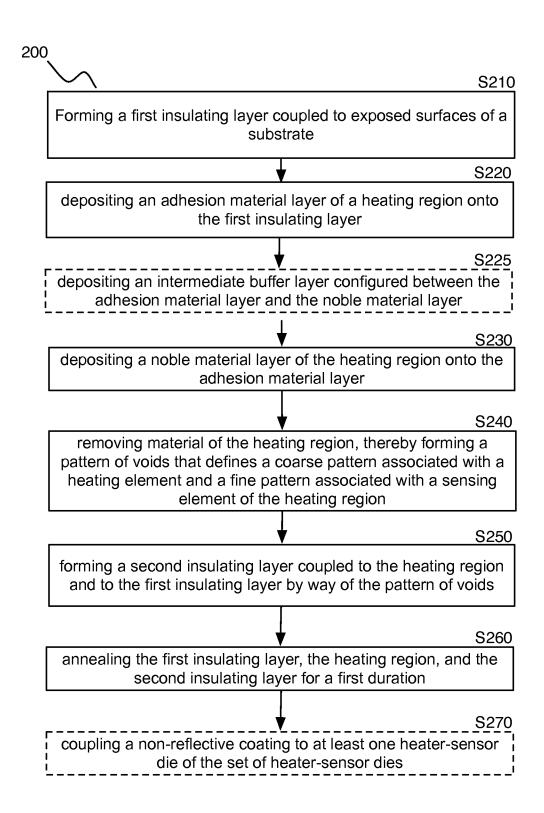


FIGURE 8

#### 1

# THERMOCYCLING SYSTEM, COMPOSITION, AND MICROFABRICATION METHOD

# CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 61/879,513 filed 18 Sep. 2013, which is incorporated in its entirety herein by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved sample thermocycling system and fabrication process thereof.

#### BACKGROUND

Molecular diagnostics is a clinical laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), 25 including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields involving analysis of nucleic acids. Molecular diagnostic analysis of biological samples can include the detection of one or more nucleic acid materials present in the specimen. 30 The particular analysis performed may be qualitative and/or quantitative. Methods of analysis typically involve isolation, purification, and amplification of nucleic acid materials, and polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample 35 to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecular diagnostic techniques are often labor/time intensive, low throughput, and expensive, and systems of analysis 40 are insufficient.

A rapid and efficient thermocycling system that can reliably thermocycle reagents used for processing of nucleic acids can significantly improve the efficiency and effective implementation of molecular diagnostic techniques, such as realtime polymerase chain reaction (RT-PCR). Microfabrication techniques can produce such thermocycling systems comprising precision heaters with low thermal masses and with well-coupled temperature sensors. However, challenges are inherent in ensuring that the microfabrication and sasembly processes utilized to fabricate thermal cycling elements are extremely robust and reliable.

Due to these challenges and deficiencies of current molecular diagnostic systems and methods, there is thus a need for an improved sample thermocycling system and 55 fabrication process thereof. This invention provides such a system and fabrication process.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A and 1B depict embodiments of a thermocycling system;

FIG. 2 depicts an example schematic of a heater-sensor die during fabrication in an example of a thermocycling system;

FIG. 3 depicts an example of heating and sensing elements in an example of a thermocycling system;

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FIGS. 4A and 4B depict examples of additional elements of an embodiment of a thermocycling system;

FIGS. 5A and 5B depict variations of configurations of elements in an embodiment of a thermocycling system;

FIGS. **6**A and **6**B depict variations of configurations of elements in an embodiment of a thermocycling system;

FIGS. 7A-7C depict variations in configurations of additional elements in an embodiment of a thermocycling system; and

FIG. **8** depicts a schematic of an embodiment of a method for manufacturing a thermocycling system.

# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of the preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

#### 1. System

As shown in FIGS. 1A and 1B, an embodiment of a system 100 for thermocycling biological samples within detection chambers comprises: a set of heater-sensor dies 110; an electronics substrate 140 that couple the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection chamber. In some embodiments, the system 100 further comprises a controller 165 and/or a cooling subsystem 170 configured to actively cool the system 100.

The system 100 functions to enable rapid thermal cycling of samples while providing uniform heating and preventing signal drift. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). The system 100 can also provide rapid thermocycling without significant power requirements, ensure a closer correlation between the actual heating temperature and the temperature set-point by implementing an integrated heatersensor die, and controllably and individually heat small sample volumes (e.g., picoliters, nanoliters) based upon a microfabrication technique that also enables mass production of the system 100. Furthermore, the system 100 implements a priori predictions of electrical resistance values of thin film combinations of the set of heater-sensor dies 110, and accounts for and/or prevents signal drift to maintain controlled sample heating. In some variations, the system 100 can be integrated into a molecular diagnostic system, such as that described in U.S. Pub. No. 2013/0210015, entitled "System and Method for Processing and Detecting Nucleic Acids", and filed on 13 Feb. 2013; however, the system 100 can additionally or alternatively be used with any other suitable system for processing biological or nonbiological samples.

#### 1.1 Heater-Sensor Dies

The set of heater-sensor dies 110 functions to controllably heat individual sample volumes. Preferably, each heater-sensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon, glass substrate) that can be packaged onto an electronics substrate 140 (e.g., printed circuit board, PCB); however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or con-

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figuration that enables controlled, uniform, and rapid heating of a detection chamber in thermal communication with the heater-sensor die 111. In some embodiments, the detection chambers can be those described in U.S. Pub. No. 2013/ 0209326, entitled "Microfluidic Cartridge for Processing 5 and Detecting Nucleic Acids" and filed on 13 Feb. 2013, which is herein incorporated in its entirety by this reference; however, the detection chambers can alternatively be any other suitable container for processing a biological sample. Preferably, each heater-sensor die 111 is characterized by a 10 small profile (e.g., <100 mm dimension), which ensures that the heater-sensor die 111 is able to thermocycle rapidly; however, a heater-sensor die 111 can alternatively be characterized by any suitable profile in order to meet any other thermocycling requirement. Additionally, each heater-sensor 15 die 111 in the set of heater-sensor dies 110 is preferably configured to conform to a detection chamber (e.g., sample tube, sample container, sample heating zone of a cartridge for processing samples) configured to contain a sample during heating; however, a heater-sensor die 111 in the set of 20 heater-sensor dies 110 can alternatively not conform to a detection chamber for processing of a biological sample.

Preferably, each heater-sensor die 111 in the set of heater sensor dies 110 comprises an assembly including: a first insulating layer 112a that functions to provide an insulating 25 barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating. Some variations can further include a second insulating layer 112b that functions to provide an additional insulating barrier that opposes the first insulating layer 112a. The insulating 30 layer(s) 112a, 112b are preferably electrically insulating, but can additionally be thermally insulating in variations where localized heating is also desired. Furthermore, each heatersensor die in preferably comprises two insulating layers 112a, 112b that are configured to "sandwich" the heating 35 region 113, thus isolating the heating region 113 at least at two surfaces of the heating region 113; however, each heater-sensor dies 111 can alternatively comprise any suitable number of insulating layers 112 arranged relative to the heating region 113 in any suitable manner. In one variation, 40 as described in further detail below, the heating region 113 can include a pattern of voids that defines elements of the heating region, and a second insulating layer 112b can be configured to couple to the heating region 113 and to the first insulating layer 112a through the pattern of voids in the 45 heating region 113, as shown in FIG. 2. The second insulating layer 112b can, however, be configured to couple to the heating region 113 and/or to the first insulating layer **112***a* in any other suitable manner.

The heating region 113 preferably comprises a heating 50 element 114 with an integrated sensing element 115, as shown in FIG. 3, and is composed of at least one metal or metal alloy, with configurations described in further detail below. Furthermore, the heating region 113 preferably defines a pattern 313 of voids having geometric features 55 (e.g., width, thickness, length, spacing) that facilitate uniform heating and provide desired heating and sensing characteristics (e.g., resistance characteristics). In some variations, the pattern 313 can comprise any one or more of: linear segments, non-linear segments, boustrophedonic seg- 60 ments, continuous segments, non-continuous segments, and any other suitable segment(s) having any other suitable geometry (i.e., width, depth, height, length, path, etc.). The pattern 313 can be symmetric about any suitable reference (e.g., reference line, reference plane, etc.), or can alterna- 65 tively be non-symmetric. Furthermore, in some variations, the pattern 313 can define a global morphology (e.g., cir-

cular footprint, ellipsoidal footprint, polygonal footprint, etc.) at a first scale (e.g., macroscopic scale) but have a local morphology at a second scale (e.g., microscopic scale), wherein the local morphology provides desired characteristics (e.g., resistance characteristics) that are attributed to elements (e.g., heating elements 114, sensing elements 115) of the heating region 113. As such, the global morphology can provide conformation (e.g., in shape) between the heating region 113 and a detection chamber coupled to or corresponding to a heater-sensor dies 111, and the local morphology can provide uniform heating and/or accurate sensing of heating parameters by utilizing structural features having a smaller governing dimension.

In a specific example, as shown in FIG. 3, the heating elements 114a, 114b, 114c with integrated sensing elements 115a, 115b, 115c are defined by a pattern 313 with a global morphology characterized by a circular footprint, but a local morphology of structures characterized by a continuous boustrophedonic arrangement, wherein both the global morphology and local morphology were designed according to theory and fabricated as described in further detail below. In the specific example, the sensing element 115 is configured to be centrally located within the global morphology of the heating element 114. However, in alternative variations, the heating region 113 can alternatively not comprise an integrated sensing element 115, can comprise any suitable number of heating elements 114/sensing elements 115, and/ or can be composed of any other suitable material.

Furthermore, during processing, each heater-sensor die 111 in the set of heater-sensor dies 110 is preferably annealed, as described in further detail below, to improve adhesion and settling of the heater-sensor materials (e.g., on a silicon substrate). The annealing can comprise a single stage of annealing or multiple stages of annealing, and can additionally or alternatively comprise different conditions (e.g., temperatures, durations, environmental conditions) during the annealing stage(s). To exemplify an effect that changes in resistance behavior can have upon heating and sensing function, a  $\sim 1-2\Omega$  deviation in resistance can cause significant deviations (e.g., 5-10° C.) between intended and actual temperatures attained by a heater-sensor dies 111. Such deviations in temperature can lead to unwanted variability in molecular diagnostic technique results and/or unreliable data (e.g., false positives, false negatives, indeterminate results). Even further, temperature swings greater than 10° C, can result in permanent damage to the thermocycling system 100 and/or to a sample being processed using the system 100. Given the importance of maintaining the calibration of the sensing elements 115 over long periods of time, it is preferable to ensure that minimal dynamics occur in the materials of the heater-sensor dies 111 after fabrication is complete. Since a majority of changes in resistance (e.g., drift) come from thermal reactions, settling, diffusion, and improved adhesion, annealing can accelerate the processes that lead to changes in resistance, thus producing heatersensor dies 111 with stable resistance behavior (e.g., stable for 3-5 years) by driving dynamic processes toward an equilibrium state (or other stable state) prior to use of the heater-sensor dies 111 in sample-processing applications. Preferably, annealing thus produces stable resistances that are substantially low (in order to enable driving at low voltages) and have limited variability, indicating that underlying material dynamics have reached equilibrium. Alternatively, during processing, each heater-sensor die 111 in the set of heater-sensor dies 110 may not be annealed, can comprise elements that limit temperature swings, and/or

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may undergo any other suitable process to produce stable resistance behavior in any other suitable manner.

In one variation, as shown in FIG. 2, the insulating layers 112a, 112b of the heater-sensor dies 111 are composed of an oxide (e.g., silicon oxide), and the heating region 113 5 comprises an adhesion material layer 116 coupled to the first insulating layer 112a and configured to promote adhesion of additional deposited layers (e.g., a noble material layer 117) of the heating region 113, and at least one noble material layer 117 coupled to the adhesion material layer 116 and configured to reduce or prevent signal drift that can result due to diffusion of the adhesion layer 116. In this variation, the first insulating layer 112a is grown or deposited uniformly on the surface of a silicon wafer (e.g., by thermal oxidation), the material layers 116, 117 of the heating region 15 113 are deposited upon the first insulating layer 112a (e.g., by evaporation, by sputtering, etc.), the heating region 113 is etched to define the heater/sensor pattern 313 of the heating element 114 and the sensing element 115 (e.g., by lithography using a positive resist, by lithography using a 20 negative resist), and a second insulating layer 112b is deposited on the heating region 113 (e.g., by chemical vapor deposition) to insulate the heating region 113 between two insulating oxide layers. Preferably, the adhesion materials used in the adhesion material layer(s) 116 comprise mate- 25 rials that are oxygen-active to react (e.g., chemically react) with an oxide surface (e.g., of an insulating layer 112a, 112b). In examples, the adhesion material layer 116 can be composed of any one or more of: chromium, titanium, niobium, vanadium and any combination or alloy thereof. In 30 examples, the noble material layer can be composed of any one or more of: gold, platinum, tungsten, palladium, and any combination or alloy thereof. Furthermore, variations of the examples can include any suitable number of adhesion material layer(s) 116 and/or noble material layer(s) 117 35 coupled between the insulating layer(s) 112a, 112b, wherein each layer 116, 117 can have any other suitable thickness.

In a first specific example of this variation, as shown in FIG. 3, a heater-sensor die 111 is configured to uniformly heat a circular region having a diameter of 5 mm, the 40 heater-sensor dies 111 has a footprint spanning a region of ~8.6 mm×7 mm, and the heating region 113 of the heatersensor dies 111 defines three heating elements 114a, 114b, 114c: a central circular heating element 114a and two circumferential heating elements 114b, 114c configured to 45 form a boundary with a serpentine-shaped pattern about the central circular heating element 114a. In the first specific example, the two circumferential heating elements 114b, 114c each form a semicircular perimeter about the central circular heating element 114a. The heater-sensor die 111 in 50 the first specific example further comprises three integrated sensing elements (i.e., resistance temperature sensors, RTDs) distributed at three locations within the 5 mm circular region. In the first specific example, the heating elements 114 comprise a 50 nm chromium adhesion material layer 55 116 and a 200 nm gold noble material layer 117 and were etched away in a boustrophedonic pattern to define the pattern of voids 313, designed using a layout editor (e.g., Mentor Graphics<sup>TM</sup> or L-Edit<sup>TM</sup>), to form the heating elements 114 and the sensing elements 115. The heating 60 elements 114a, 114b, 114c are defined by coarse patterning in the first specific example, and the sensing elements 115 are integrated with the heating elements 114a, 114b, and 114c and defined by fine patterning, as shown in FIGS. 3A and 3B. In the first specific example, the heating elements 65 114 are characterized with resistances in the range of 40-100Ω and the accompanying sensing elements 115 (i.e.,

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RTDs) are characterized with resistances in the range of  $200\text{-}250\Omega$  prior to annealing. With annealing at  $400~\mathrm{F}$  in an inert  $N_2$  atmosphere, the resistances for both the heating elements 114 and the sensing elements 115 increased 400-600% after 1 hour, and increased up to 800% with increasing anneal times.

In other embodiments of the heater-sensor dies 111, the heating element(s) 114 and/or the sensing element(s) 115 can comprise other combinations of adhesion material layer(s) 116 and/or noble material layer(s) 117 in addition to or alternative to chromium and gold, including any one or more of: titanium (adhesion), platinum (noble), tungsten (noble), and any combination or alloy thereof. In particular, chromium and titanium can serve as preferable adhesion materials for an active noble layer being coupled to the adhesion material layer 116, due to attributes that enable them to react with oxide materials. The combination of chromium and gold additionally can undergo processing by a lift-off method or an etching process to form a heating element 114 and/or a sensing element 115, as described in further detail in Section 2 below. While in some applications it can be difficult to etch platinum films due to platinum's non-reactivity, platinum has a preferable temperature coefficient of resistance (TCR) of  $\sim 0.00385\Omega/\Omega/C$  that makes it stable and preferable for RTD fabrication. Platinum and/or titanium can even serve as an intermediate buffer layer 119, configured between an adhesion material layer 116 and a noble material layer 117, that can prevent diffusion from an adhesion material layer 116 into a noble material layer 117 and stabilize resistance behavior (e.g., electrical resistance dynamics). As such, variations of the heating region 113 can include any suitable number of intermediate buffer layers 119 that prevent diffusion between an adhesion material layer 116 into an adjacent noble material layer 117.

In a second specific example, the heating elements 114 comprise a 50 nm chromium adhesion material layer 116, an 100 nm platinum intermediate buffer layer 119 coupled to the adhesion material layer 116, and a 300 nm gold noble material layer 117 coupled to the intermediate buffer layer 119 and were etched away in an identical pattern to that in the first specific example to form a surface including the heating and sensing elements 114, 115. Similar to the first specific example, the heating elements 114 of the second specific example are defined by coarse patterning, and the sensing elements 115 are defined by fine patterning and integrated into the coarse patterning of the heating elements 114. Also similar to the first specific example, the heating region 113 is configured between two insulating layers 112a, 112b in the second specific example. In the second specific example, the heating elements 114 were characterized to have resistances of approximately  $50\Omega$  and the sensing elements were characterized to have resistances around  $130\Omega$  prior to annealing. After annealing at 400 F in an inert N<sub>2</sub> atmosphere, the resistances for both the heating elements 114 and the sensing elements increased 10-30% after 1 hour, with no substantial further change in resistance following additional annealing of 2 hours.

In a third specific example, the heating elements 114 comprise a 50 nm chromium adhesion material layer 116 and a 100 nm platinum noble material layer 117 coupled to the adhesion material layer 116 and were etched away in an identical pattern to that in the first specific example, to form a surface including the heating and sensing elements 114, 115. Similar to the first specific example, the heating elements 114 of the third specific example are defined by coarse patterning, and the sensing elements 115 are integrated with the coarse patterning of the heating elements 114 and

removal

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defined by fine patterning. Also similar to the first specific example, the heating region 113 is configured between two insulating layers 112a, 112b in the third specific example. In the third specific example, the heating elements 114 are characterized with resistances around  $2\Omega$  prior to annealing. 5 After annealing at 400 F in an inert  $N_2$  atmosphere, the resistances for the heating elements 114 decreased 7% after 1 hour, with no substantial change in resistance following additional annealing of 2 hours.

In a fourth specific example, the heating elements 114 10 comprise a 50 nm titanium/tungsten adhesion material layer 116 and a 440 nm gold noble material layer 117 and were etched away in an identical pattern to that in the first specific example, to form a surface including the heating and sensing elements 114, 115. Similar to the first specific example, the 15 heating elements 114 are defined by coarse patterning, and the sensing elements 115 are integrated with the coarse patterning of the heating elements 114 and defined by fine patterning. Also similar to the first specific example, the heating region 113 is configured between two insulating 20 layers 112a, 112b in the fourth specific example. In the fourth specific example, the heating elements 114 are characterized with resistances around  $40\Omega$  and the sensing elements 115 are characterized with resistances around  $100\Omega$  prior to annealing. After annealing at 400 F in an inert 25  $N_2$  atmosphere, the resistances for both the heating elements 114 and the sensing elements 115 decreased 11-14% after 1 hour, with no substantial further change in resistance following additional annealing of 2 hours.

In the second, third, and fourth specific examples, analysis of the resistance values pre-annealing and post-annealing indicated that the compositions and processing of the respective thin film layers 112, 116, 117 significantly stabilize the resistance values and dynamics of resistance behavior of the heating and sensing elements 114, 115 after annealing. The 35 third specific example with chromium and platinum layers demonstrated substantially no variance from pre-anneal measurements of the electrical resistance after the second annealing step, and the fourth specific example with titanium, tungsten, and gold layers demonstrated a 11%-14% 40 reduction in resistance following the first annealing step but then showed no additional variance after the second annealing step. No further changes in resistance following stages of annealing indicate that the dynamic behavior of the materials has been arrested or completed (e.g., has reached 45 equilibrium). The second specific example with chromium, platinum, and gold layers demonstrated a slightly higher variance (~10%-30%) from pre-anneal resistance values indicating a higher level of dynamic behavior in these thin films, and further annealing was not completely sufficient to 50 arrest the dynamics with this combination. However, the combination of different adhesion and noble materials with the intermediate buffer layer(s) 119 to reduce or eliminate diffusion between adhesion layers 116 and noble layers 117, along with the annealing stage(s) produced positive effects 55 with regard to stability of resistance behavior.

Other variations and examples of the heater-sensor dies 111 can comprise any suitable patterning configuration, any suitable arrangement of insulating layer(s) 112 and heating region(s) 113, any suitable combination of adhesion, buffer, 60 and/or noble layers 116, 119, 117, and/or any suitable annealing or other process that facilitates stabilization of resistance behavior in the set of heater-sensor dies 110. 1.1.1 Heater-Sensor Theory

In the specific examples described above, the microfabricated heater-sensor dies **111** of the set of heater-sensor dies **110** are configured in a manner that produces rapid thermo8

cycling for a given level of power input. Design of the pattern 313 of heater-sensor dies 111 in the specific examples was performed using a layout editor (e.g., Mentor Graphics<sup>TM</sup> or L-Edit<sup>TM</sup>), in order to generate the boustrophedonic structures of the local morphology of the heater elements 114, within the bounds of the global circular morphology of the heater elements 114. Estimates of the electrical resistance of both thin-film heating elements 114 and sensing elements 115 (e.g., RTDs) of the specific examples was performed based upon a determination of sheet resistances of the thin film layer(s) of the adhesion and noble layers 116, 117 being deposited upon the insulating layer 112, with thicknesses of the thin film layers ranging from 50 nm-500 nm. In the specific example, sheet resistances were calculated based on the resistivity of the thin film and the width, thickness, and length of the thin film regions of the adhesion and noble layers 116, 117 being deposited. As such, in the specific examples, the layouts of the heating and sensing element 114, 115 patterns are configured to obtain desired heater-sensor coverage on a region (e.g., a region proximal to a surface of a sample container) being thermally cycled. In the specific examples, the configuration of the heater-sensor dies 111 also ensures that electrical resistance values of the heater-sensor dies are in within a desirable range, from an energy-use standpoint (e.g., a range that enables a range of temperatures with a low input voltage).

Theoretically, the resistance(s) of the heating element(s) 114 are preferably in the range of  $50\text{-}100\Omega$  to enable efficient heating of a substrate with an input voltage from 20-30 volts; however, the heating elements 114 can alternatively be characterized by other resistance ranges. A heating element 114 design characterized by a resistance between  $50\text{-}100\Omega$  and that uses an input voltage of 20-30 volts provides a dissipation of 4-18 Watts of heat and is capable of rapidly heating a desired region of a thermally conductive substrate (e.g., silicon substrate). In addition, such a design parameters can produce a temperature across a  $1 \text{ cm} \times 1 \text{ cm}$  substrate that equilibrates and produces a uniform temperature profile in a diffusion time of less than 1 second. Such a diffusion time thus enables well-controlled biochemical reactions, with regard to temperature uniformity and control.

Theoretically, the resistance(s) of the sensing element(s) 115 (e.g., RTD) are preferably in the range  $200\text{-}300\Omega$ , based upon correlating a "change in resistance" (dependent upon TCR) against the temperature of the sensing element 115. In one variation, the resistance behavior of an RTD can be characterized by the following equation:

 $R=R0*(1+\alpha*\Delta T+\beta*\Delta T^2)$ 

where R=Resistance of an RTD at an unknown temperature (T); R0=Resistance of temperature at a reference temperature T0 (e.g., room temperature);  $\alpha$  and  $\beta$ =experimentally determined constants; and  $\Delta$ T=Difference in temperature (T-T0). Under this equation, the change in resistance ( $\Delta$ R) for an RTD per degree change ( $\Delta$ T=1) is given by  $\Delta$ R=( $\alpha$ + $\beta$ )\*R0.

As such, for efficient temperature sensing as well as ensuring a detected temperature resolution of 0.1-0.2 C using RTDs, the resistance change per degree change in temperature is preferably greater than  $\sim$ 0.1 $\Omega$ . While higher changes in resistance can be desired, such higher changes in temperature, for a given RTD material or thin film combination, results only when the initial resistance (R0) of the RTD is substantially high. However, having a substantially high initial resistance increases the risk of the RTD self-heating during the resistance sensing process, thereby poten-

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tially causing additional noise/disturbances and inaccuracies in temperature measurement. The RTDs in a specific example of the system **100** were calibrated by measuring the resistance against 4-5 temperature points in the range the sensor is intended to be utilized. For biochemical assays, the 5 typical range of temperatures is room temperature (25° C.) to ~100° C. By characterizing the resistance (R) at each of several temperatures (T) within a range of intended operation, one can obtain an experimental relationship for R vs. T. 1.2 Other System Elements

As shown in FIGS. 1, 4A-4B, and 7A-7C, the system 100 can further comprise an electronics substrate 140 configured to couple heating and sensing elements of the set of heatersensor dies to a controller 165, a set of heat-sink supports 150 configured to facilitate heat dissipation within the 15 system 100, a set of elastic elements 160 configured to bias the set of heater-sensor dies 110 against detection chambers for sample processing, and can additionally comprise the controller 165 and/or a cooling subsystem 170.

The electronics substrate 140 is preferably coupled to the 20 set of heater-sensor dies 110, and functions to enable communication between heating elements 114 and sensing elements 115 of each heater-sensor die 111 in the set of heater-sensor dies no and a controller 165. As such, the electronics substrate preferably enables communication of 25 heat output commands from the controller 165 to the heating element(s) 114, and communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 to the controller 165. The electronics substrate 140 preferably comprises a printed circuit board 30 (PCB), and in one variation is a flexible PCB, as shown in FIG. 4A, in order to facilitate contact between heater-sensor dies 111 in the set of heater-sensor dies 110 and detection chambers (e.g., reaction vessels, sample containers, etc.) for processing of a sample according to molecular diagnostic 35 protocols. Alternatively, the PCB can be a substantially rigid PCB or any other suitable PCB.

Preferably, the set of heater-sensor dies 110 is coupled to the electronics substrate 140 in a manner that provides thermal and/or electrical isolation of each heater-sensor dies 40 111 from the neighboring heater-sensor die(s) due to poor conductivity of the electronics substrate 140. However, the electronics substrate 140 and the set of heater-sensor dies 110 can be configured in any alternative suitable manner that provides isolation of each heater-sensor die 111. In some 45 variations, each heater-sensor die 111 is coupled to the electronics substrate by an adhesive layer 141 and/or a wire bond 142, as shown in FIG. 5A; however, each heater-sensor dies 111 can alternatively or additionally be coupled to the electronics substrate 140 in any suitable manner (e.g., using 50 solder bonds in flip-chip bonding), as shown in FIG. 5B. Furthermore, the system 100 can comprise any suitable number of electronics substrates.

The set of heat sink supports 150 is preferably coupled to at least one of the set of heater-sensor dies 110 and the 55 electronics substrate 140 and functions to facilitate rapid thermocycling by dissipating heat from the set of heater-sensor dies 110 and/or the electronics substrate 140. The set of heat sink supports 150 can further function to provide structural support for the set of heater-sensor dies 110, such 60 that the set of heater-sensor dies 110 is supported during compression (e.g., compression against a set of detection chambers) and/or tension. In the absence of heat sinking, the electronics substrate 140 and the surrounding environment can potentially retain too much heat, which compromises the 65 cooling of the set of heater-sensor dies 110. The set of heat sink supports 150 can comprise multiple heat sink supports

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151 configured to define any suitable number of contact locations, or can alternatively comprise a single heat sink support 151 configured to define any suitable number of contact locations. As shown in FIGS. 6A and 6B, the system 100 preferably couples to a detection chamber (e.g., reaction vessel, detection chamber) at a first side 101a of the detection chamber, as shown in FIG. 4A, which can restrict heat dissipation from the first side 101a of the system. Furthermore, the second side 101b of the detection chamber is typically used for optical imaging for monitoring (e.g., realtime monitoring, delayed monitoring), and further limiting heat-sinking from the second side 101b. Thus, it is preferable for the set of heat sink supports 150 to couple to the system 100 from a side of the system 100 that does not physically interfere with optical imaging apparatus interfacing with the system 100. However, alternative configurations of the set of heat sink supports 150 can comprise coupling at any suitable side and/or any number of sides of the system 100.

As shown in FIGS. 6A and 6B, the set of heat sink supports 150 can be configured in any of a number of variations. In a first variation, each heat sink support 151 can be directly placed against a first surface 105a of heatersensor die 111 opposing that of a second surface 105b contacting a detection chamber, as shown in FIG. 6A. The first variation enables efficient transfer of heat out of the first surface 105a of the heater-sensor die away from a respective detection chamber; however, excessive heat sinking can affect heating ramp rates. In a second variation, the system 100 comprises a thermally insulating assembly 152 between a heater-sensor die 111 and a corresponding heat sink support 151, as shown in FIG. 6B. In the second variation, the electronics substrate 140 can serve as the thermally insulating assembly 152 and can be situated between the heater-sensor die 111 and a heat sink support 151. Furthermore, in the second variation, a suitable thermal resistance provided by the electronics substrate 140 (e.g., through thickness, material selection, a combination of features) could produce a thermal couple between the heater-sensor dies 111 and the heat sink support 151 to permit the heating capacity of the heater-sensor die 111 to achieve the heating times and/or heating ramp rate required by the application, while still allowing adequate cooling rates. Additionally, the second variation can provide increased backside support to each of the set of heater-sensor dies 110 as well as increased surface for adhesion.

In specific examples of the second variation, heat sinking and supporting the "backside" of the electronics substrate 140 can be implemented across multiple heater-sensor dies 111, separated by Society for Laboratory Automation and Screening (SLAS) standard spacings, such as 9 mm, 4.5 mm or 2.25 mm spacings. The heat sink support 151 material (e.g., aluminum, copper, silver) in the specific examples is mated with the electronics substrate 140 at each heatersensor die location, with an air gap positioned laterally between each heater-sensor die location. This configuration can further function to reduce cross talk across a set of detection chambers in contact with the set of heater-sensor dies 110. The set of heat sink supports 150 can, however, be configured in any other suitable manner to provide heat dissipation within the system 100, without obstruction of optical detection apparatus, and with provision of desired heat ramping and/or cycling behavior.

The set of elastic elements 160 is preferably coupled to a first surface 104a of the electronics substrate 140, as shown in FIG. 4A, and functions to promote contact between the set of heater-sensor dies 110 and detection chambers (e.g.,

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reaction vessels, detection chambers) for sample processing according to molecular diagnostic protocols. The set of elastic elements 160 can comprise any one more of springs and elastomeric elements, which can deform and provide transmit a biasing force, through the electronics substrate 5 140, to reinforce contact between a set of detection chambers and the set of heater-sensor dies 110. The set of elastic elements 160 can, however, additionally or alternatively include any other suitable elements configured to provide a biasing force that reinforces contact between a set of detection chambers and the set of heater-sensor dies 110 in an elastic or a non-elastic manner. In one such alternative variation, the system 100 can include one or more actuators configured to drive each of the set of heater-sensor dies 111 toward a corresponding detection chamber, and in another 15 such alternative variation, the system 100 can include a set of magnets (e.g., including magnet pairs surrounding the set of heater-sensor dies 110 and a corresponding set of detection chambers), that function to reinforce coupling between the set of heater-sensor dies 110 and the set of detection 20 chambers. However, any other suitable elements can additionally or alternatively be used to facilitate uniform and consistent coupling between the set of heater-sensor dies  $110\,$ and a set of detection chambers.

In embodiments of the system 100 including a set of 25 elastic elements 160, the set of elastic elements 160 is preferably coupled to a first surface 104a of the electronics substrate 140, such that each elastic element in the set of elastic elements 160 facilitates contact between a heatersensor dies 111 and a corresponding detection chamber. In a 30 first variation, the set of elastic elements 160 is coupled to first surface 104a of a flexible PCB of the electronics substrate 140, as shown in FIG. 4A. In the first variation, contact between each heater-sensor dies 111 and a corresponding detection chamber is thus maintained by a biasing 35 force provided by an individual spring through the flexible PCB of the electronics substrate 140. In the first variation, the number of elastic elements in the set of elastic elements 160 is equal to the number of heater-sensor dies in the set of heater-sensor dies 110, such that the set of elastic elements 40 160 and the set of heater-sensor dies 110 are paired in a one-to-one manner. Alternatives to the first variation can, however, comprise any suitable number of elastic elements in relation to a number of heater-sensor dies 110. In a second variation, the set of heater-sensor dies 110 is coupled to a 45 second surface 104b of a rigid PCB of the electronics substrate 140, with the set of elastic elements 160 coupled to the first surface 104a of the rigid PCB. In the second variation, the set of elastic elements 160 thus functions to collectively transfer a force through the rigid PCB to main- 50 tain contact between the set of heater-sensor dies 110 and the detection chambers. Alternatives to the second variation can also comprise any suitable number of springs in relation to a number of heater-sensor dies in the set of heater-sensor dies 110. Furthermore, variations of the system 100 can 55 include one or more elastic elements coupled to any other elements directly or indirectly coupled to the set of heatersensor dies 110. For instance, the system 100 can additionally or alternatively include one or more springs 160' coupled to base surfaces of the set of heat-sink supports 150 60 interfacing with the set of heater-sensor dies, in order to transmit biasing forces.

As shown in FIGS. 1A and 1B, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of 65 heater-sensor dies 110. The controller 165 can further be configured to provide heat parameter output commands to

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the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100. The controller 165 preferably comprises a proportionintegral-derivative (PID) controller, but can alternatively be any other suitable controller 165. The controller 165 preferably interfaces with the set of heater-sensor dies 110 through the electronics substrate 140 by a connector; however, the controller 165 can interface with the set of heatersensor dies 110 in any alternative suitable manner. Preferably, the controller 165 is configured to automate and control heat output parameters, including any one or more of: heating temperatures, heating ramp rates, heating times (e.g., holding times), and any other suitable heating parameter(s). Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies 110. In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistance-to-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control. In another specific example, the controller 165 comprises a National Instruments LabView based system comprised of an NI cDAQ-9178 chassis with an NI 9219 universal analog input card and an NI 9485 eight-channel solid-state relay sourcing or sinking digital output module solid-state relay card. In this specific example, the cDAQ-9178 supports the NI 9219 and NI 9485 cards, the NI 9219 is used to obtain the RTD inputs, and the NI 9485 cycles the power supply voltage to individual heater-sensor dies of the set of heater-sensor dies 110. Further, in this specific example, the controller 165 is expandable to 12 or more channels through the use of additional NI 9219 and NI 9485 cards, each of which can handle several channels.

As shown in FIGS. 8A and 8B, the system 100 can further comprise a cooling subsystem 170, which functions to provide heat transfer from the system 100 in order to further enhance controlled heating and cooling by the system 100. The cooling subsystem 170 is preferably configured to provide at least one of convective cooling and conductive cooling of the system 100, but can alternatively be configured to provide any other suitable cooling mechanism or combination of cooling mechanisms. In one variation, the cooling subsystem 170 can comprise a fan 171 that provides convective heat transfer from the system 100. In this variation, the fan 171 can be coupled to any suitable element of the system 100, such as the set of heat sink supports 150, as shown in A. Furthermore, alternatives to this variation can comprise any suitable number of fans of any suitable dimension and configuration, examples of which are shown in FIGS. 7A and 7B. In one such example, the system can include a set of cooling elements integrated with each heat sink support of the set of heat sink supports. In another variation, the cooling subsystem 170 can additionally or alternatively comprise a Peltier device, as shown in FIG. 7C. The Peltier device can be cooled and maintained at a defined temperature (e.g., in the 10-25 C range) to provide a

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substantial temperature gradient for cooling during a thermal cycling process, which can decrease cooling times and/or cycle times. In yet another variation, the cooling subsystem 170 can additionally or alternatively comprise a liquid cooling system (e.g., water cooling system) configured to 5 surround and absorb heat from one or more heater-sensor dies of the set of heater-sensor dies 110, for instance, by way of the set of heat sink supports 150. The cooling subsystem 170 can additionally or alternatively comprise any other suitable cooling element(s).

In some variations, reflection from the set of heater-sensor dies 110 can interfere with light transmitted to photodetectors of an optical subsystem opposed (e.g., directly opposed, in opposition) to the set of heater-sensor dies 110 (e.g., light emitted from the set of biological samples, light transmitted 15 through filters of an optical subsystem), especially in configurations wherein a set of detection chambers is configured between the set of heater-sensor dies and optical elements of an optical subsystem. In these variations, the set of heatersensor dies 110 can include elements that reduce or elimi- 20 nate reflection from any portion of the set of heater-sensor dies (e.g., reflection from the heating region, etc.), thereby facilitating analysis of a set of biological samples within the set of detection chambers. In one variation, the set of heater-sensor dies 110 can include or be coupled to one or 25 more non-reflective coatings 180 at surfaces of the set of heater-sensor dies 110 upon which light from the optical subsystem impinges. In a specific example, the non-reflective coating 180 can comprise a high-temperature paint (e.g., dark paint, flat paint) that functions to absorb and/or diffuse 30 light from an opposing optical subsystem, while facilitating heat transfer to a set of detection chambers in thermal communication with the set of heater-sensor dies 110. In another variation, the set of heater-sensor dies 110 can be configured to be in misalignment with photodetectors of the 35 optical subsystem, such that reflection does not interfere with light transmitted to the photodetectors of the optical subsystem. In one example, the set of heater-sensor dies 110 can be configured to heat a set of detection chambers from a first direction, and the optical subsystem can be configured 40 to receive light from the set of detection chambers from a second direction (e.g., a direction non-parallel to the first direction), such that reflection from the set of heater-sensor dies 110 does not cause interference. In still other variations, the set of heater-sensor dies 110 can include any other 45 suitable elements (e.g., coatings, layers, etc.) and/or be configured in any other suitable manner that eliminates. prevents, or mitigates reflection from the set of heater-sensor dies 110 from interfering with light transmitted to photodetectors of an optical subsystem in opposition to the set of 50 heater-sensor dies 110.

Variations of the system 100 can, however, include any other suitable element(s) configured to provide uniform, accurate, precise, and reliable heating of one or more detection chambers in thermal communication with the system 55 100. Furthermore, as a person skilled in the art will recognize from the previous detailed description and from the figures, modifications and changes can be made to the preferred embodiments of the system 100 without departing from the scope of the system 100.

#### 2. Method of Manufacture

As shown in FIG. **8**, a method **200** of manufacturing a system for thermocycling biological sample within detection chambers comprises: forming a first insulating layer coupled to exposed surfaces of a substrate S**210**, depositing an 65 adhesion material layer of a heating region onto the first insulating layer of the substrate S**220**, depositing a noble

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material layer of the heating region onto the adhesion material layer S230; removing material of the heating region S240, thereby forming a pattern of voids that defines a coarse pattern associated with a heating element of the heating region and a fine pattern, integrated into the coarse pattern and associated with a sensing element of the heating region; forming a second insulating layer coupled to the heating region and to the first insulating layer by way of the pattern of voids S250; and annealing the first insulating layer, the heating region, and the second insulating layer for a first duration S260.

The method 200 functions to generate heating and sensing elements of a thermocycling system that can provide rapid thermocycling without significant power requirements, while ensuring a suitably close correlation between an actual heating temperature and a temperature set-point (i.e., an intended heating temperature). In some embodiments, the method 200 can function to produce a heating and sensing element of an integrated heater-sensor die as described in Section 1 above, which can controllably and individually heat small sample volumes (e.g., picoliters, nanoliters, microliters). Furthermore, the method 200 of manufacture preferably implements a priori predictions of electrical resistance values of thin film combinations of the set of heatersensor dies 110, as described in Section 1 above, and accounts for and/or prevents signal drift in relation to actual and intended heating temperatures, to maintain controlled sample heating.

Block S210 recites: forming a first insulating layer coupled to exposed surfaces of a substrate, which functions to electrically isolate portions of a heating region from other elements of the thermocycling system. The substrate is preferably a silicon substrate, but can alternatively be any other suitable semi-conducting, or non-conducting substrate. As such, in variations, the substrate can be composed of a semi-conducting material (e.g., silicon, quartz, gallium arsenide), and/or an insulating material (e.g., glass, ceramic). In some variations, the substrate 130 can even comprise a combination of materials (e.g., as in a composite, as in an alloy). In examples wherein the substrate is a silicon substrate, the substrate can be composed of silicon with any suitable type (e.g., P-type), doping (e.g., boron-doping), miller index orientation, resistivity, thickness, total thickness variation, and/or polish.

In forming the first insulating layer, Block S210 can be performed using any one or more of: thermal oxide growth, chemical vapor deposition (CVD), spin coating, spray coating, and any other suitable method of depositing a localized layer of an insulating material. Preferably, the first insulating layer is composed of an insulating oxide material, and in examples can include any one or more of: a thermally grown silicon oxide, a chemical vapor deposited oxide, a deposited titanium oxide, a deposited tantalum oxide, and any other suitable oxide grown and/or deposited in any other suitable manner. However, the first insulating layer can additionally or alternatively include an insulating polymer (e.g., a polyimide, a cyanate ester, a bismaleimide, a benzoxazine, a phthalonitrile, a phenolic, etc.) that is chemical and heat resistant and/or any other suitable material (e.g., chemical 60 vapor deposited nitride, other nitride, paralene, etc.) that is configured to provide the first insulating layer.

In one example of Block S210, the first insulating layer comprises an oxide material, and is formed by growing the oxide material on a substrate. In one example of Block S210, the insulating layer comprises a 0.2 mm layer of silicon oxide, and is formed on a 100 mm silicon wafer using thermal oxidation at 900° C. using water vapor (i.e., in wet

oxidation) or oxygen (i.e., in dry oxidation) as the oxidant. In alternative variations and examples of Block S210, the first insulating layer can be formed using high or low temperature thermal oxidation, using any suitable oxidant,

temperature thermal oxidation, using any suitable oxidant, and/or using any other suitable method (e.g., fluid deposition of an electrically insulating polymer, softbaking/hardbaking of a deposited polymer, etc.).

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Block S220 recites: depositing an adhesion material layer of a heating region onto the first insulating layer of the substrate, which functions to facilitate bonding of a noble 10 material layer of the heating region to the first insulating layer as in Block S230. The adhesion material preferably comprises an adhesion material that is characterized as active in reacting with the first insulating layer, in order to facilitate coupling between the first insulating layer and the adhesion material. However, the adhesion material of the adhesion material layer can additionally or alternatively have any other suitable characteristic(s). In variations wherein the first insulating layer comprises an insulating oxide, the adhesion materials used in the adhesion material 20 layer(s) can comprise materials that are oxygen-active to chemically react with an oxide surface (e.g., materials that have a high heat of oxide formation). In examples of oxygen-active materials, the adhesion material layer can be composed of any one or more of: chromium, titanium, 25 niobium, vanadium, any other suitable adhesion material that reacts with the insulating layer, and any combination or alloy of any of the above materials.

In Block S220, the adhesion material layer is preferably coupled to the first insulating layer by one or more of 30 evaporation and sputtering. However, in alternative variations, the adhesion material layer can be coupled to the first insulating layer by any one or more of: deposition (e.g., electrodeposition, CVD, etc.), plating (e.g., chemical plating, electroplating), and any other suitable process of cou- 35 pling the adhesion material layer to the first insulating layer. Furthermore, in examples wherein the adhesion material layer is evaporated or sputtered, the insulating layer-substrate subassembly generated in Block S210 can be translated or rotated in order to facilitate uniform deposition of 40 the adhesion material layer. In Block S220, the adhesion material layer is preferably processed to a thickness of under 100 nm; however, the adhesion material layer can alternatively be processed to any other suitable thickness. In specific examples, the adhesion material layer comprises a 45 50 nm thick layer of chromium, or a 50 nm thick layer of a combination of titanium and tungsten.

Block S230 recites: depositing a noble material layer of the heating region onto the adhesion material layer, which functions to form a portion of a heating region comprising 50 a heating element and a sensing element of the system. The noble material preferably comprises a noble material that is characterized as having good thermal conductivity and affinity to coupling with the adhesion material layer in a reliable manner during thermocycling of the system. The noble 55 material layer can alternatively have characteristics including any one or more of: characteristics that inhibit diffusion between the adhesion material layer and the noble material layer, high fatigue resistance, high fracture resistance, and any other suitable property that provides reliability during 60 thermocycling of the system. In examples of noble materials that operate well with examples of adhesion materials described above, the noble material layer can be composed of any one or more of: gold, platinum, tungsten, palladium, any other noble material that interacts well with the adhesion 65 material layer, and any combination or alloy of any of the above materials.

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In Block S230, the noble material layer is preferably coupled to the adhesion material layer by one or more of evaporation and sputtering. However, in alternative variations, the noble material layer can be coupled to the adhesion material layer by any one or more of: deposition (e.g., electrodeposition, CVD, etc.), plating (e.g., chemical plating, electroplating), and any other suitable process of coupling the noble material layer to the adhesion material layer. Furthermore, in examples wherein the noble material layer is evaporated or sputtered, the adhesion material layerinsulating layer-substrate subassembly generated in Block S220 can be translated or rotated in order to facilitate uniform deposition of the noble material layer. In Block S230, the noble material layer is preferably processed to a thickness of under 500 nm; however, the noble material layer can alternatively be processed to any other suitable thickness. In specific examples, the noble material layer comprises a 200 nm thick layer of gold, a 300 nm thick layer of gold, a 440 nm thick layer of gold, and/or a 100 nm thick layer of platinum.

In some variations, the method 200 can further include Block S225, which recites: depositing an intermediate buffer layer configured between the adhesion material layer and the noble material layer. Block S225 functions to provide a layer that reduces or prevents diffusion between the noble material layer and the adhesion material layer, thus improving dynamic stability (e.g., in electrical resistance behavior) of the system. In variations, the intermediate buffer layer thus has characteristics that contribute to behavior as a barrier against diffusion between the adhesion material layer and the noble material layer. In examples, the intermediate buffer layer comprises platinum and/or titanium, and can be processed onto an adhesion material layer by one or more of evaporation and sputtering. However, in alternative variations, the intermediate buffer layer can be coupled to the adhesion material layer by any one or more of: deposition (e.g., electrodeposition, CVD, etc.), plating (e.g., chemical plating, electroplating), and any other suitable process of coupling the intermediate buffer layer to adhesion material layer, prior to coupling of noble material layer to the intermediate buffer layer. The intermediate buffer layer is preferably processed to a thickness of less than 150 nm, and in specific examples can comprise a 100 nm thick layer of platinum, or a 50 nm thick layer of titanium. However, the intermediate buffer layer can alternatively have any other suitable thickness. Furthermore, variations of the heating region 113 comprising adhesion material layers and noble material layers can include any suitable number of adhesion material layers, noble material layers, and intermediate buffer layers that prevent diffusion between an adhesion material layer into an adjacent noble material layer, wherein each layer can have any other suitable thickness.

Block S240 recites: removing material of the heating region, which functions to form a pattern of voids that defines a coarse pattern associated with a heating element of the heating region and a fine region, integrated into the coarse pattern and associated with a sensing element of the heating region. Block S240 functions to produce elements of the heating region with desired resistance behavior and characteristics, in order to provide reliable and accurate heat parameter sensing and output within the system, Block S240 preferably provides one or more heating elements and sensing elements integrated with the heating elements, wherein the heating element(s) and the sensing element(s) have desired theoretical resistance characteristics as described in Section 1.1.1 above; however, Block S240 can

17 alternatively provide heating elements and sensing elements with any other suitable resistance characteristics.

Block S240 preferably produces a pattern of voids having geometric features (e.g., width, thickness, length, spacing) that facilitate uniform heating and provide desired heating 5 and sensing characteristics (e.g., resistance characteristics). In some variations, the pattern of voids can define any one or more of: linear segments, non-linear segments, boustrophedonic segments, continuous segments, non-continuous segments, and any other suitable segment(s) having any 10 other suitable geometry (i.e., width, depth, height, length, path, etc.) within an assembly of the adhesion material layer(s), noble material layer(s), and/or intermediate buffer layer(s) produced in Blocks S220, S230 and/or S225. The pattern of voids produced in Block S240 can further be 15 symmetric about any suitable reference (e.g., reference line, reference plane, etc.), or can alternatively be non-symmetric. Furthermore, in some variations, the pattern of voids can define a global morphology (e.g., circular footprint, ellipsoidal footprint, polygonal footprint, etc.) at a first scale 20 (e.g., macroscopic scale) but have a local morphology at a second scale (e.g., microscopic scale), wherein the local morphology provides desired characteristics (e.g., resistance characteristics) that are attributed to elements (e.g., heating elements, sensing elements) of the heating region. As such, 25 the global morphology can provide conformation (e.g., in shape) between the heating region and a detection chamber configured to contact the heating region, and the local morphology can provide uniform heating and/or accurate sensing of heating parameters by utilizing structural features 30 having a smaller governing dimension.

The pattern of voids is preferably defined entirely through the assembly of the adhesion material layer(s), noble material layer(s), and/or intermediate buffer layer(s) produced in Blocks S220, S230 and/or S225 (e.g., through the assembly 35 to the first insulating layer); however, the pattern of voids can alternatively be defined to any other suitable thickness through the assembly. Preferably, Block S240 includes producing the pattern of voids by way of a lithographic process process with a negative resist). However, producing the pattern of voids in Block S240 can additionally or alternatively be performed using any one or more of: etching, punching, die-cutting, water cutting, laser cutting, and any other suitable method of removing material. In one example, 45 the assembly comprising the adhesion material layer and the noble material layer produced in examples of Blocks S220 and S230 can be covered with positive photoresist (e.g., a photomask designed according to the heating pattern) and lithographically etched in exposed regions. In the example, 50 the positive photoresist can then be removed to reveal the pattern of voids. In other variations of the example, the pattern of voids can be produced using any lithographic method, using positive and/or negative etching to form the heating pattern, and/or using any other suitable method. In 55 one example of an alternative implementation of Step S240', the pattern of voids can be produced using a lift-off process, wherein a sacrificial layer is used to define the pattern of voids, the heating region material(s) is (are) deposited, and then the sacrificial layer is removed to reveal the heating 60

In a specific example of Block S240, an assembly comprising an adhesion material layer, a noble material layer, and an intermediate buffer layer configured between the adhesion material layer and the noble material layer is 65 configured to have a global footprint defining circular region having a diameter of 5 mm. The pattern of voids, produced

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by a lithographic process, defines three heating elements including: a central circular heating element and two circumferential heating elements, as shown in FIG. 3, configured to form a boundary with a serpentine-shaped pattern about the central circular heating element. In the specific example, the two circumferential heating elements are each configured to form a semicircular perimeter about the central circular heating element. The pattern of voids produced in the specific example of Block S240 further comprises three integrated sensing elements (i.e., resistance temperature sensors, RTDs) distributed at three locations within the 5 mm circular footprint. In the specific example, the pattern of voids was designed using a layout editor (e.g., Mentor Graphics™ or L-Edit™), to define the heating elements and the sensing elements according to desired resistance characteristics as described in Section 1.1.1 above. The specific example of Block S240 produced heating elements having coarse patterning, and sensing elements 115 integrated with the heating elements and having fine patterning, as shown in FIGS. 3A and 3B. Variations of the specific example of Block S240 can, however, generate any other suitable pattern of voids to define the heating element(s) and the sensing element(s) in any other suitable manner.

Blocks S210, S220, S230, and S240 are preferably performed in the order described; however, Blocks S210, S220, S230, and S240 can alternatively be performed in any other suitable order. For instance, in one variation the patterned assembly comprising the adhesion material layer(s), the noble material layer(s), and/or the intermediate buffer layer(s) can be formed by any suitable method (e.g., molding, laser cutting, stamping, 3D printing, stereolithography, etc.) and then coupled to the first insulating layer according to any suitable manner.

Block S250 recites: forming a second insulating layer coupled to the heating region and to the first insulating layer by way of the pattern of voids, which functions to electrically isolate portions of a heating region from other elements of the thermocycling system, and to further provide stability within the system. In forming the second insulating layer, (e.g., lithographic process with a positive resist, lithographic 40 Block S250 is preferably performed using a deposition process (e.g., CVD); however, Block S250 can additionally or alternatively be performed using any one or more of: thermal oxide growth, spin coating, spray coating, and any other suitable method of depositing a localized layer of an insulating material. Preferably, the second insulating layer is composed of an insulating oxide material, and in examples can include any one or more of: a silicon oxide (e.g., deposited by CVD, thermally grown), a titanium oxide, a tantalum oxide, and any other suitable oxide grown and/or deposited in any other suitable manner. However, the first second layer can additionally or alternatively include an insulating polymer (e.g., a polyimide, a cyanate ester, a bismaleimide, a benzoxazine, a phthalonitrile, a phenolic, etc.) that is chemical and heat resistant and can be processed onto the first insulating layer and the heating region by any one or more of: casting (e.g., drop casting), printing, dipping, and using any other suitable method. The second insulating layer processed in Block S250 can additionally or alternatively comprise any other suitable material (e.g., chemical vapor deposited nitride, other nitride, paralene, etc.) that is configured to provide the second insulating layer.

> Block S260 recites: annealing the first insulating layer, the heating region, and the second insulating layer for a first duration, which functions to stabilize dynamic resistance behavior of heating and sensing elements of the system. Since a majority of changes in resistance (e.g., drift) come from thermal reactions, settling, diffusion, and improved

how does pattern of voids define heating elements

methods of removing material

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adhesion, annealing can accelerate the processes that lead to changes in resistance, thus producing a system with stable resistance behavior (e.g., stable for 3-5 years) by driving dynamic processes toward an equilibrium state (or other stable state) prior to use of the system in sample-processing applications. In Block S260, annealing can comprise a single stage of annealing or multiple stages of annealing, and can additionally or alternatively comprise different conditions (e.g., temperatures, durations, environmental conditions) during the annealing stage(s). Preferably, annealing thus 10 produces stable resistances that are substantially low (in order to enable driving of the system at low voltages) and have limited variability, indicating that underlying material dynamics have reached equilibrium.

In Block S260, annealing is preferably performed at 15 constant temperature, and in variations, can be performed at a temperature between 300 C and 600 C (or any other suitable temperature below a melting temperature of the noble material layer and the adhesion material layer); however, annealing can alternatively be performed at any other 20 suitable temperature or range of temperatures, and can additionally or alternatively be performed at a non-constant temperature (e.g., cyclically varying temperature, non-cyclically varying temperature). Annealing is preferably performed in an inert atmosphere (e.g., inert N<sub>2</sub> atmosphere) to 25 control reactions between the atmosphere and elements being annealed; however, annealing can alternatively be performed in any other suitable atmosphere. In a specific example, Block S240 includes annealing the first insulating layer, the heating region, and the second insulating layer at 30 400 F in an inert N<sub>2</sub> atmosphere for one hour, which can, in some variations of the specific example, be extended for up to 2 hours following the first hour of annealing.

As shown in FIG. 8, the method 200 can further include Block S270, which recites: coupling a non-reflective coating 35 to at least one heater-sensor die of the set of heater-sensor dies. Block S270 functions to process at least a subset of the set of heater-sensor dies 110 so that they do not interfere with light transmitted to photodetectors of an optical subsystem opposed (e.g., directly opposed, in opposition) to the 40 set of heater-sensor dies 110 (e.g., light emitted from the set of biological samples, light transmitted through filters of an optical subsystem), especially in configurations wherein a set of detection chambers is configured between the set of heater-sensor dies and optical elements of an optical sub- 45 system. The non-reflective coating is preferably coupled identically to all heater-sensor dies of the set of heater sensor dies; however, the non-reflective coating can alternatively be coupled non-identically to one or more heater-sensor dies of the set of heater-sensor dies. As such, in variations, one or 50 more subsets of the set of heater-sensor dies can be coupled to non-reflective coatings in a manner that provides different light reflection properties for the subset(s) of the set of heater-sensor dies.

In Block S270, the non-reflective coating is preferably a 55 material layer that is applied superficial to at least one of the first insulating layer and the second insulating layer processed in variations of Blocks S210 and S250, respectively. In one example, the non-reflective coating processed in Block S270 can comprise a high-temperature paint (e.g., 60 dark paint, flat paint) that functions to absorb and/or diffuse light from an opposing optical subsystem, while facilitating heat transfer to a set of detection chambers in thermal communication with the set of heater-sensor dies. In this example, the high-temperature paint can be applied by any 65 one or more of: brushing, spraying, dipping, printing, and any other suitable method of coupling the high-temperature

of heater-sensor dies. However, the non-reflective coating can alternatively be processed simultaneously with or can comprise one or more of the first insulating layer and the second insulating layer processed in variations of Blocks S210 and S260, respectively. For instance, one or more of the first and the second insulating layer can include a modified oxide layer that has low-reflectivity, thus preventing interference caused by light reflected from the set of heater-sensor dies. In some extreme variations, however, mitigation of interference due to reflected light from the set of heater-sensor dies can be produced by configuring the set of heater-sensor dies to be in misalignment with photodetectors of the optical subsystem, such that reflection does not interfere with light transmitted to the photodetectors of the optical subsystem, in modified versions of Block S270. For instance, the set of heater-sensor dies can be configured to heat a set of detection chambers from a first direction, and the optical subsystem can be configured to receive light from the set of detection chambers from a second direction (e.g.,

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paint to one or more surfaces of at least a subset of the set

reflection from the set of heater-sensor dies 110 does not cause interference. In still other variations of Block S270, the set of heater-sensor dies can be processed with any other suitable elements (e.g., coatings, layers, etc.) and/or be configured in any other suitable manner that eliminates, prevents, or mitigates reflection from the set of heater-sensor dies from interfering with light transmitted to photodetectors of an optical subsystem in opposition to the set of heater-sensor dies 110.

The method 200 can additionally or alternatively include any other suitable blocks or steps configured to facilitate

a direction non-parallel to the first direction), such that

The method 200 can additionally or alternatively include any other suitable blocks or steps configured to facilitate fabrication of a thermocycling element that can heat small volumes (e.g., microliter volumes, nanoliter volumes, picoliter volumes) of biological samples within containers in contact with the thermocycling element. For instance, the method 200 can include any one or more of: coupling the heating region to an electronics substrate S280; coupling the electronics substrate to an elastic element configured to bias the system against a detection chamber S290; and coupling at least one of the electronics substrate and the heating region to a heat sink-support S300 that facilitates heat dissipation within the system, while providing mechanical support for the heating region within the system. The method 200 can, however, include any other suitable blocks or steps.

The system 100 and/or method 200 of the preferred embodiment and variations thereof can be embodied and/or implemented at least in part as a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system 300 and one or more portions of the processor 350. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instructions.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of methods according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, seg-

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ment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, 5 two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following 20 claims.

We claim:

- 1. A system for thermocycling biological samples within detection chambers comprising:
  - a set of heater-sensor dies, each heater-sensor die in the 25 set of heater-sensor dies comprising:
    - an assembly including a first insulating layer, a heating region comprising an adhesion material layer coupled to the first insulating layer and a noble material layer coupled to the adhesion material layer, and a second insulating layer coupled to the heating region and to the first insulating layer through a pattern of voids in the heating region,
    - wherein the pattern of voids in the heating region defines a coarse pattern, comprising a global morphology at a first scale and associated with a heating element of the heating region, and a fine pattern, comprising a local morphology at a second scale smaller than the first scale, integrated into the coarse pattern and associated with a sensing element of the heating region;
  - an electronics substrate configured to couple heating elements and sensing elements of the set of heatersensor dies to a controller; and
  - a set of elastic elements coupled to a second substrate <sup>45</sup> surface of the electronics substrate opposing a first substrate surface of the electronics substrate interfacing with the assemblies of the set of heater-sensor dies and configured to bias each of the set of heater-sensor dies against a detection chamber in a configuration wherein

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- the set of heater-sensor dies is in thermal communication with a set of detection chambers.
- 2. The system of claim 1, wherein the adhesion material layer comprises at least one of chromium, titanium, niobium, and vanadium, and the noble material layer comprises at least one of gold, platinum, tungsten, and palladium.
- 3. The system of claim 1, wherein the adhesion material layer and the noble material layer are annealed layers configured to provide stabilized resistance behavior and prevent drift in heating and sensing using the heating region.
- 4. The system of claim 2, wherein the assembly further comprises an intermediate buffer layer composed of at least one of platinum and titanium, situated between the adhesion material layer and the noble material layer, and configured to provide a barrier against diffusion between the adhesion material layer and the noble material layer.
- 5. The system of claim 2, wherein the adhesion material layer has a thickness less than or equal to 100 nm, and the noble material layer has a thickness less than or equal to 500 nm.
- 6. The system of claim 2, wherein the pattern of voids defines a pattern of boustrophedonic segments including wide segments of the coarse pattern associated with the heating element and segments of the fine pattern, surrounded by and narrower than the wide segments, associated with the sensing element of the heating region.
- 7. The system of claim 6, wherein the pattern defined through the adhesion material layer and the noble material layer is configured to provide a resistance change per temperature change greater than 0.1 ohms/° C.
- 8. The system of claim 7, wherein the electronics substrate is a flexible substrate and wherein the system further comprises a set of heat-sink supports coupled to at least one of the set of heater-sensor dies and the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies, wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element configured to transmit a biasing force through the flexible substrate, thereby maintaining thermal communication between the set of heater-sensor dies and the set of detection chambers in the configuration wherein the set of heater-sensor dies is in thermal communication with the set of detection chambers.
- **9**. The system of claim **1**, wherein each of the set of heater-sensor dies includes a coating, proximal the heating surface, configured to mitigate reflection of light from the heating surface toward photodetectors of an optical subsystem, in a configuration wherein the set of heater-sensor dies is opposed to photodetectors of the optical subsystem.

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# EXHIBIT 29

## (12) United States Patent

#### Brahmasandra et al.

#### US 9,539,576 B2 (10) Patent No.:

#### (45) Date of Patent: \*Jan. 10, 2017

#### (54) THERMOCYCLING SYSTEM AND MANUFACTURING METHOD

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(2006.01)B01L 3/00 (2006.01)B01L 7/00 (2006.01)

(52) U.S. Cl.

CPC ...... B01L 7/52 (2013.01); B01L 2200/12 (2013.01); B01L 2200/147 (2013.01); (Continued)

Field of Classification Search

(58)

CPC ...... B01L 7/525; B01L 2300/1822; B01L 2300/1827; B01L 3/502723; B01L 7/52

See application file for complete search history.

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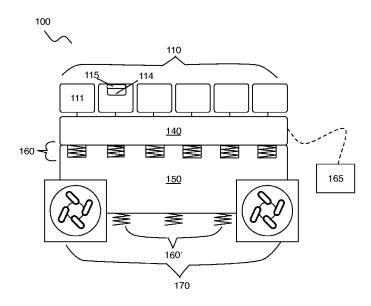
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#### ABSTRACT

A system for thermocycling biological samples within detection chambers comprising: a set of heater-sensor dies, each heater-sensor die comprising a heating surface configured to interface with a detection chamber and a second surface, inferior to the heating surface, including a first connection point; an electronics substrate, comprising a first substrate surface coupled to the second surface of each heater-sensor die, an aperture providing access through the electronics substrate to at least one heater-sensor die, and a second substrate surface inferior to the first substrate surface, wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface. an aperture surface defined within the aperture, and the second substrate surface, and wherein the electronics substrate is configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; and a set of wire bonds, including a wire bond coupled between the first connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.

#### 7 Claims, 10 Drawing Sheets



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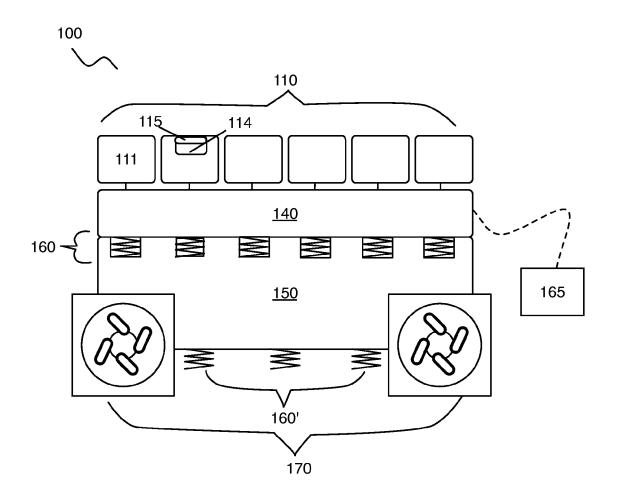
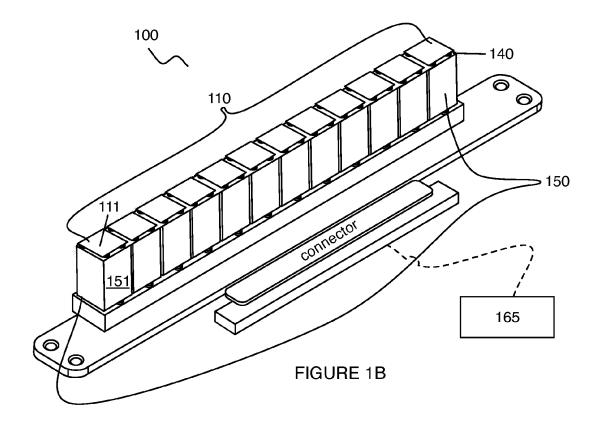
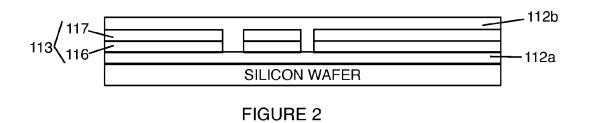


FIGURE 1A

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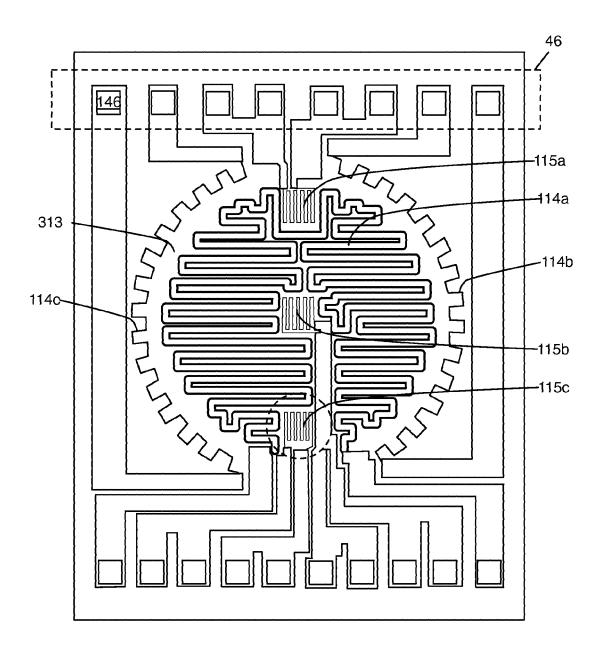
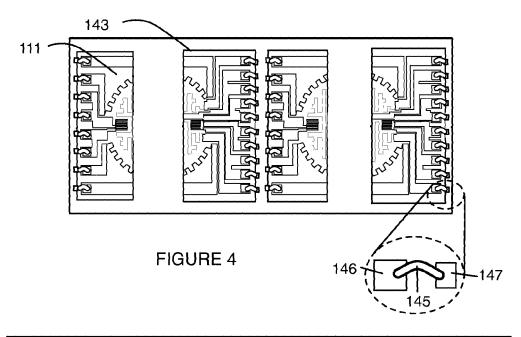


FIGURE 3

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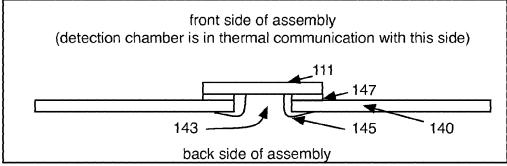


FIGURE 5A

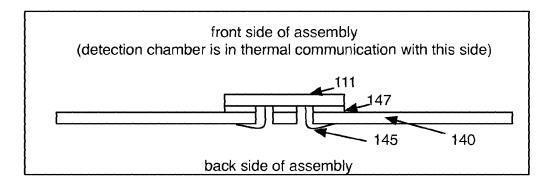
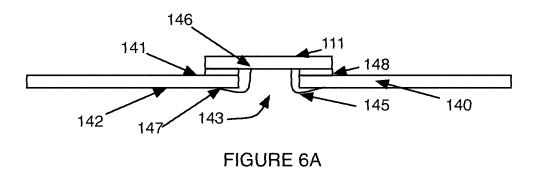
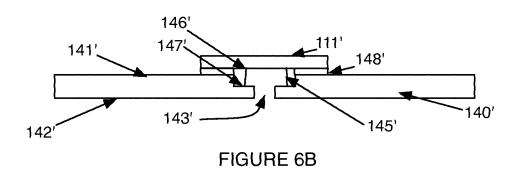


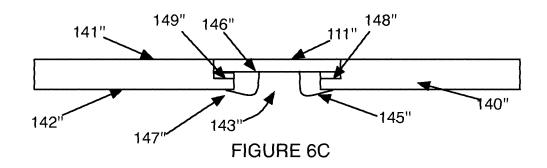
FIGURE 5B

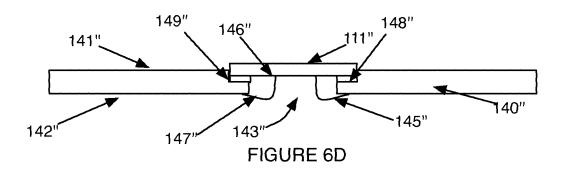
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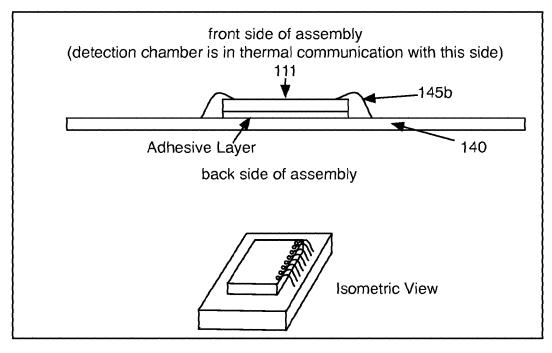


FIGURE 7A

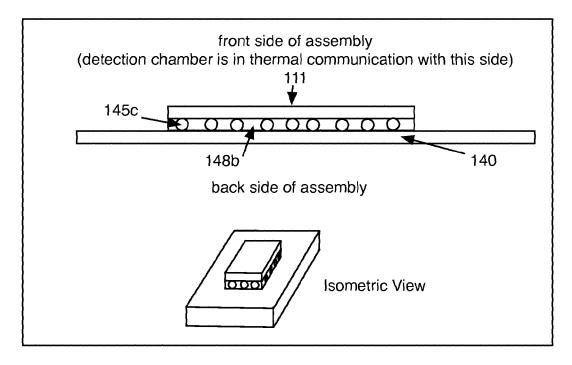
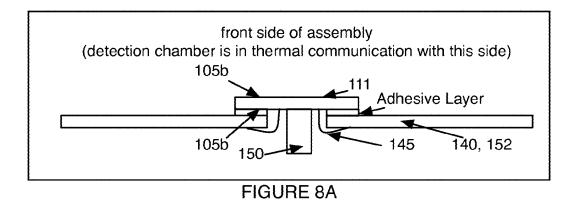


FIGURE 7B

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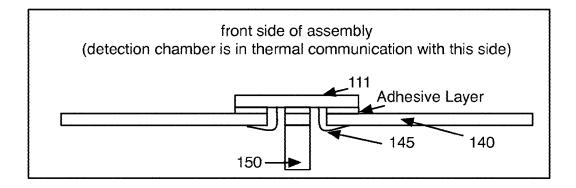


FIGURE 8B

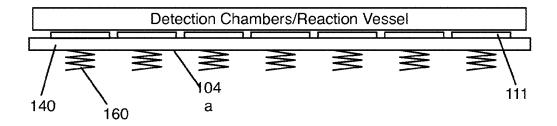
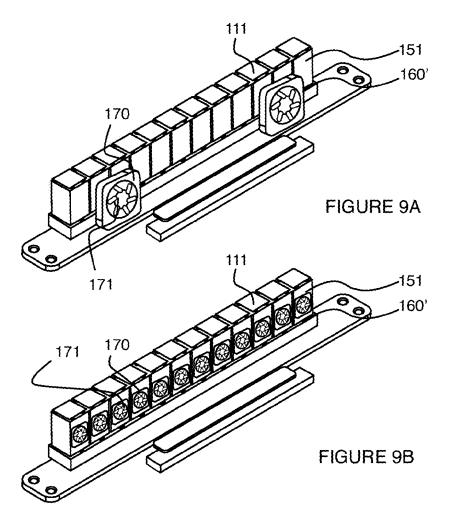


FIGURE 8C

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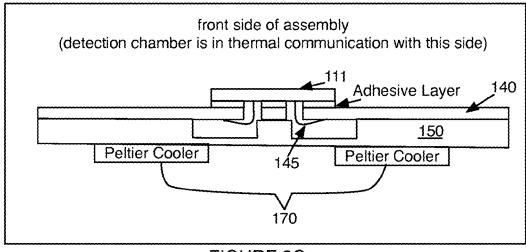


FIGURE 9C

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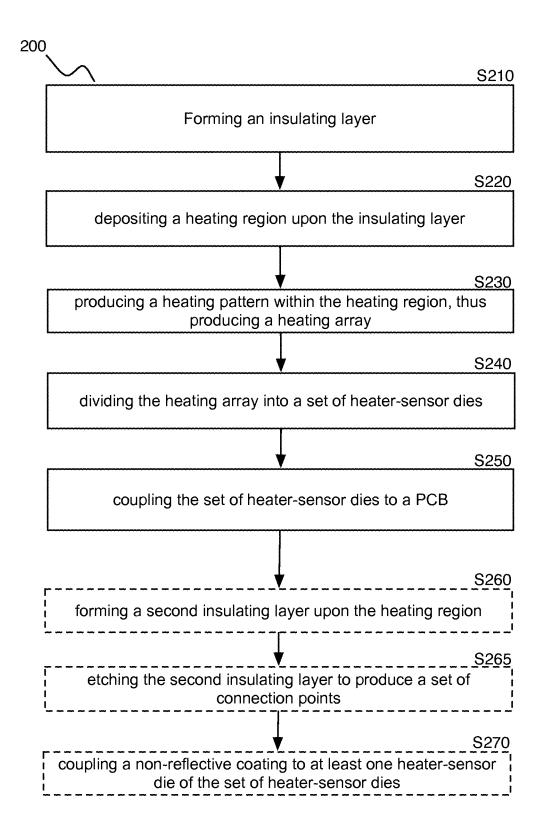
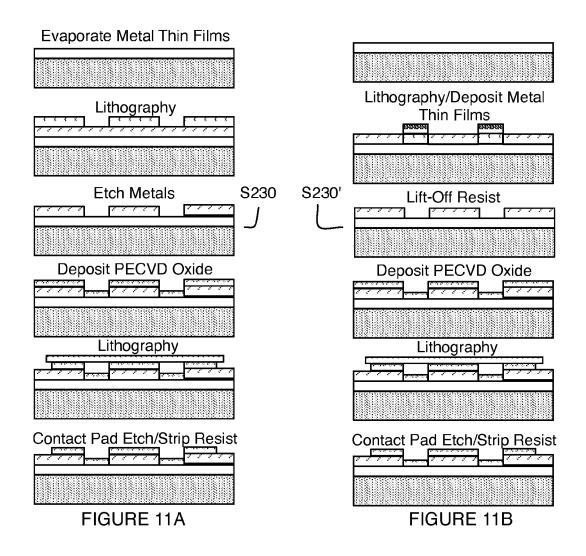


FIGURE 10

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#### THERMOCYCLING SYSTEM AND MANUFACTURING METHOD

#### CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 61/879,517 filed 18-Sep.-2013, which is incorporated in its entirety herein by this reference. This application is also related to U.S. application Ser. No. 14/487,808 filed 16-Sep.-2014, which is incorporated herein in its entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved sample thermocycling system and assembly method thereof.

#### BACKGROUND

Molecular diagnostics is a clinical laboratory discipline that has developed rapidly during the last 25 years. It research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields involving analysis of nucleic acids. Molecular diagnostic 30 analysis of biological samples can include the detection of one or more nucleic acid materials present in the specimen. The particular analysis performed may be qualitative and/or quantitative. Methods of analysis typically involve isolation, purification, and amplification of nucleic acid materials, and 35 polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and 40 molecular diagnostic techniques are often labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient.

A rapid and efficient thermocycling system that can reliably thermocycle reagents used for processing of nucleic 45 acids can significantly improve the efficiency and effective implementation of molecular diagnostic techniques, such as realtime polymerase chain reaction (RT-PCR). Microfabrication techniques can produce such thermocycling systems comprising precision heaters with low thermal masses and 50 with well-coupled temperature sensors. However, challenges are inherent in ensuring that the microfabrication and assembly processes utilized to fabricate thermal cycling elements are extremely robust and reliable.

Due to these challenges and deficiencies of current 55 molecular diagnostic systems and methods, there is thus a need for an improved sample thermocycling system and assembly method thereof. This invention provides such a system and assembly method.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A and 1B depict embodiments of a thermocycling system;

FIG. 2 depicts an example schematic of a heater-sensor 65 die during fabrication in an example of a thermocycling

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FIG. 3 depicts an example of heating and sensing elements in an example of a thermocycling system;

FIG. 4 depicts examples of additional elements of an embodiment of a thermocycling system;

FIGS. 5A and 5B depict examples of reverse wire bonding in embodiments of a thermocycling system;

FIGS. 6A-6D depict examples of reverse wire bonding in embodiments of a thermocycling system;

FIGS. 7A-7B depict variations of configurations of elements in an embodiment of a thermocycling system;

FIGS. 8A-8C depict variations of configurations of elements in an embodiment of a thermocycling system;

FIGS. 9A-9C depict variations of configurations of additional elements in an embodiment of a thermocycling sys-

FIG. 10 depicts a flowchart of a method for assembling an embodiment of a thermocycling system; and

FIGS. 11A-11B depict variations of a method for assembling an embodiment of a thermocycling system.

#### DESCRIPTION OF THE PREFERRED **EMBODIMENTS**

The following description of the preferred embodiments originated from basic biochemistry and molecular biology 25 of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

1. System

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As shown in FIGS. 1A and 1B, an embodiment of a sample thermocycling system 100 comprises: a set of heatersensor dies 110; an electronics substrate 140 configured to couple heating elements and sensing elements of the set of heater-sensor dies to a controller; a set of heat sink supports 150 coupled to at least one of the electronics substrate and the set of heater-sensor dies; and a set of elastic elements 160 coupled to the electronics substrate and configured to bias each of the set of heater-sensor dies against a detection chamber. In some embodiments, the system 100 further comprises a controller 165 coupled to the electronics substrate and configured to automate and/or control relevant heating parameters of the system 100, and can further comprise a cooling subsystem 170 configured to dissipate heat from the system 100. The system 100 functions to enable rapid thermocycling of samples while providing uniform heating and preventing mechanical failure of the system 100 during thermocycling. In specific applications, the system 100 can be used to rapidly and controllably thermocycle nucleic acid samples during performance of molecular diagnostic amplification techniques (e.g., PCR, RT-PCR), signal amplification techniques (e.g., bDNA, hybrid capture), and analytical techniques (e.g., gel electrophoresis, mass spectrometry). In some variations, the system 100 can be integrated into a molecular diagnostic system, such as that described in U.S. Pub. No. 2013/0210015, entitled "System and Method for Processing and Detecting Nucleic Acids", and filed on 13 Feb. 2013; however, the system 100 can additionally or alternatively be used with any other suitable system for processing biological or nonbiological samples.

60 1.1 Heater-Sensor Dies

The set of heater-sensor dies no functions to controllably heat individual sample volumes. Preferably, each heatersensor die 111 is a thin-film die that can be deposited onto another substrate (e.g., silicon substrate, glass substrate) that can be packaged onto an electronics substrate 140; however, each heater-sensor die 111 can alternatively comprise any suitable geometry and/or configuration that enables con-

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trolled, uniform, and rapid sample heating of a detection chamber in thermal communication with the heater-sensor die 111. In some embodiments, the detection chambers can be those described in U.S. Pub. No. 2013/0209326, entitled "Microfluidic Cartridge for Processing and Detecting 5 Nucleic Acids" and filed on 13 Feb. 2013, which is herein incorporated in its entirety by this reference; however, the detection chambers can alternatively be any other suitable container for processing a biological sample. Preferably, each heater-sensor die 111 is characterized by a small profile 10 (e.g., <10 mm dimension), which ensures that the heatersensor die 111 is able to thermocycle extremely rapidly; however, a heater-sensor dies 111 can alternatively be characterized by any suitable profile. Additionally, each heatersensor die 111 is preferably configured to conform to a 15 detection chamber (e.g., sample tube, sample container, sample heating zone of a cartridge for processing samples) configured to contain a sample during heating; however, a heater-sensor die 111 in the set of heater-sensor dies 110 can alternatively not conform to a sample container. In one 20 variation, each heater-sensor die 111 can be coupled to a thermally conductive element (e.g., 600 micron×5×5 mm silicon spacer) using thermally conductive grease or another suitable material. In this variation, a connection between a heater-sensor die is thus protected against failure due to 25 shear forces that can result from placement of a sample container on a heater-sensor die 111. Other variations of preventing connection failure are described in Section 1.2 below.

Preferably, each heater-sensor die 111 in the set of heater 30 sensor dies no comprises an insulating layer 112 that functions to provide an insulating barrier to isolate the heaters and sensors and a heating region 113 that functions to provide uniform sample heating, as shown in FIG. 2. The insulating layer 112 is preferably electrically insulating, but 35 can additionally be thermally insulating. Furthermore, each heater-sensor die 111 preferably comprises two insulating layers 112 that are configured to "sandwich" the heating region 113, thus isolating the heating region 113; however, each heater-sensor die 111 can alternatively comprise any 40 suitable number of insulating layers 112 arranged relative to the heating region 113 in any suitable manner. The heating region 113 preferably comprises a heating element 114 with an integrated sensing element 115, and is composed of a metal or metal alloy. Furthermore, the heating region 113 is 45 preferably defined by a pattern defined by geometric features (e.g., width, thickness, length, spacing) that facilitate uniform heating. However, in variations, the heating region 113 can alternatively not comprise an integrated sensing element 115, can comprise any suitable number of heating elements 50 114/sensing elements 115, and/or can be composed of any other suitable material.

In a first specific example of a heater-sensor die 111, as shown in FIG. 3, a heater-sensor die 111 is configured to uniformly heat a circular region having a diameter of 5 mm, 55 spans a region of ~8.6 mm×7 mm, and comprises three heating elements 114a, 114b, 114c: a central circular heating element 114a and two circumferential heating elements 114b, 114c configured to form a boundary about the central circular heating element. The heater-sensor die 111 in the 60 first specific example further comprises three integrated sensing elements 115a, 115b, 115c (i.e., resistance temperature sensors, RTDs) distributed at three locations within the 5 mm circular region. In the first specific example, the heating region was 113 etched away in a boustrophedonic 65 pattern, designed using a layout editor (e.g., Mentor Graphics<sup>TM</sup> or L-Edit<sup>TM</sup>), to form the heating surface. The heating

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elements 114 are defined by coarser patterning, and the sensing elements 115 are defined by finer patterning, as shown in FIG. 3A. Other variations and examples of the heater-sensor dies 111 can comprise any suitable patterning configuration and/or any suitable arrangement of insulating layer(s) 112a, 112b and heating region(s) 113.

1.2 Other System Elements

As shown in FIGS. 1A and 1B, the system 100 further comprises an electronics substrate 140 and a set of heat-sink supports 150. Furthermore, the system 100 can additionally comprise a controller 165 configured to automate and/or control relevant heating parameters of the system 100, and/or a cooling subsystem 170 configured to dissipate heat from the system 100, as shown in FIGS. 9A and 9B.

The electronics substrate 140 is preferably coupled to the set of heater-sensor dies 110, and functions to enable communication between each heater-sensor die 111 in the set of heater sensor dies 110 and a controller 165. The electronics substrate 140 preferably comprises a printed circuit board (PCB), and in some variations, comprises a flexible PCB, as shown in FIG. 4, in order to facilitate contact between heater-sensor dies 111 in the set of heater-sensor dies 110 and detection chambers (e.g., reaction vessels, detection chambers) for processing according to molecular diagnostic protocols. Alternatively, the electronics substrate 140 can alternatively comprise a rigid PCB or any other suitable PCB. Furthermore, the system 100 can comprise any suitable number of PCBs. Preferably, the set of heater-sensor dies 110 is assembled onto the electronics substrate in a manner that provides thermal and/or electrical isolation of each die in from the neighboring die(s), in particular, for variations wherein the electronics substrate 140 is characterized as having poor conductivity. However, the electronics substrate 140 and the set of heater-sensor dies no can be configured in any alternative suitable manner that provides isolation of each die in.

Preferably, the electronics substrate 140 is configured to couple to a heater-sensor die 111 by a reverse wire bond 145 coupled between a first connection point 146 (i.e., contact pad) of a set of connection points 46 on the heater-sensor die 111 and a second connection point 147 (i.e., pad) on the electronics substrate 140, as shown in FIGS. 4 and 5A-5B. The reverse wire bond 145 functions to prevent unbonding of a heater-sensor die 111 from the electronics substrate 140 that can result from shear forces on at least one of the heater-sensor die 111 and the wire bond 145 and/or fatigue of the wire bond 145 during thermocycling. The reverse wire bond 145 can be made from a back-side of the electronics substrate 140, in the orientation shown in FIGS. 5A-5B, through an aperture 143 defined within the electronics substrate 140. The aperture 143 can be a single aperture, or a set of apertures corresponding to the set of heater-sensor dies 110, and furthermore, multiple heater-sensor dies 111 of the set of heater-sensor dies 110 can be configured to couple to the electronics substrate 140 through an aperture 143 defined within the electronics substrate 140. As such, the mappings between pads on the heater-sensor dies no and the electronics substrate 140 can be one-to-one or many-to-one in variations of coupling. In one variation, a set of apertures can be longitudinally spaced across the electronics substrate 140; however, in other variations, the set of apertures can be distributed across the electronics substrate 140 in any other suitable manner. Also shown in FIGS. 5A-5B, coupling between the electronics substrate 140 and a heater-sensor die 111 can additionally comprise an adhesive layer 148 comprising cyanoacrylate and/or any other suitable adhesive material configured between the electronics substrate 140

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and the heater-sensor die 111. In variations of heater-sensor die 111 coupling to the electronics substrate 140 with an adhesive layer 148, the adhesive layer is preferably heat resistance in order to prevent failure at the adhesive layer 148 during thermocycling.

In a first variation, as shown in FIG. 6A, a heater-sensor die 111 is configured to couple to a first side 141 of the electronics substrate 140 by a wire bond 145 that passes through an aperture 143 defined within the electronics substrate 140, such that the wire bond 145 couples at one end to a second side 142 of the electronics substrate 140. In this variation, a first connection point 146 on a surface of the heater-sensor die 111 closer to the first side 141 of the electronics substrate 140 is coupled to a second connection point 147 on the second side of the electronics substrate 140, 15 by way of the aperture 143 and the reverse wire bond 145. In the first variation, the heater-sensor die 111 can be further stabilized in place by an adhesive layer 148 located between the first side 141 of the electronics substrate 140 and the heater-sensor die 111. Furthermore, while one wire bond 145 20 is described, the electronics substrate 140 can include a set of connection points distributed at regions of the second substrate surface between adjacent apertures of a set of apertures of the electronics substrate 140.

In a second variation, as shown in FIG. 6B, a heatersensor die 111' is configured to couple to a first side 141' of the electronics substrate 140' by a wire bond 145' that passes into an aperture 143' defined within the electronics substrate 140. In this variation, the wire bond 145 couples, at a first connection point 146', to a surface of the heater-sensor die 30 111 closer to the first side 141' of the electronics substrate 140' and terminates at a second connection point 147' part-way between a first side 141' and a second side 142' of the electronics substrate 140', such that the wire bond 145' is not exposed at the second side of the electronics substrate 35 140'. In the second variation, the heater-sensor die 111' can also be stabilized in place by an adhesive layer 148' located between the first side 141' of the electronics substrate 140' and the heater-sensor die.

In a third variation, as shown in FIGS. 6C and 6D, a 40 heater-sensor die 111" is configured to rest within a recess 149" at the first side of the electronics substrate 140", wherein the recess 149" is connected to (e.g., contiguous with) an aperture 143" defined within the electronics substrate 140". As shown in FIG. 6C, the recess 149" can be 45 configured such that a heating surface of the heater-sensor die 111 is flush with a first surface 141" of the electronics substrate 140"; however, in an example shown in FIG. 6D, the recess can also be configured such that a heating surface of the heater-sensor die 111" is not flush with the first surface 50 141" of the electronics substrate 140". In the third variation, the wire bond 145" couples, at a first connection point 146" to a surface of the heater-sensor die 111" partially situated within the electronics substrate 140", and terminates at a second connection point 147" either part-way between the 55 first side 141" and a second side 142" of the electronics substrate 140" (as in the second variation), or at a second point 147" at the second side 142" of the electronics substrate 140" (as in the first variation). Thus, in the third variation, a surface of the heater-sensor die 111" is stabilized 60 within the recess 149" of the electronics substrate 140" to further prevent shearing or other forms of mechanical failure that could compromise coupling between the heater-sensor die 111" and the electronics substrate 140". In the third variation, the heater-sensor die 111" can also be further 65 stabilized within the recess 149" by an adhesive layer 148" between the recess 149" and the heater-sensor die 111".

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While a single recess is described, the electronics substrate 140" can include a set of recess, each contiguous with at least one aperture of set of apertures of the electronics substrate 140.

Other variations of the reverse wire bond(s) 145 between a heater-sensor die 111 and the electronics substrate 140 can comprise any suitable combination of the above variations, and can additionally or alternatively comprise any suitable encapsulation, embedding, or potting of wire bonds to further prevent failure in the wire bonds.

In still other variations, each heater-sensor die 111 can be coupled to the electronics substrate 140 by any other suitable method. In one variation, the coupling can comprise a "top-side" wire bond 145b, as shown in FIG. 7A. In this variation, a thin wire (e.g., 10-300 microns thick) composed of an electrically conductive material (e.g., aluminum, gold, or copper wire) is coupled between a first connection point 146 on the heater-sensor die 111 and a second connection point 147 on a second side 142 of the electronics substrate 140, in the orientation shown in FIG. 7A. Furthermore, in this variation, the "top-side" wire bonds 145b are potted, embedded, and/or encapsulated to protect them from mechanical failure. In another variation, the coupling can comprise a flip-chip bond 145c, as shown in FIG. 7B. In this variation, a volume (e.g., ball) of solder is placed between a first connection point 146 on a heater-sensor die 111 and a second connection point 147 on the electronics substrate 140. Furthermore, in this variation, a filler material 148b can be placed in regions between the electronics substrate 140 and the heater-sensor die 111 not connected by a volume of solder of the flip-chip bond 145c. In other variations, the coupling can additionally or alternatively comprise any suitable adhesive (e.g., cyanoacrylate adhesive) layer 148 configured between the heater-sensor die 111 and the electronics substrate 140.

The set of heat sink supports 150 is preferably coupled to at least one of the set of heater-sensor dies no and the electronics substrate 140 and functions to facilitate rapid thermocycling by dissipating heat from the set of heatersensor dies 110 and/or the electronics substrate 140. The set of heat sink supports 150 can further function to provide structural support for the set of heater-sensor dies 110, such that the set of heater-sensor dies no is supported during compression (e.g., compression against a set of detection chambers) and/or tension. In the absence of heat sinking, the electronics substrate 140 and the surrounding environment can potentially retain too much heat, which compromises the cooling of the set of heater-sensor dies 110. The set of heat sink supports 150 can comprise multiple heat sink supports 151 configured to define any suitable number of contact locations, or can alternatively comprise a single heat sink support 151 configured to define any suitable number of contact locations. As shown in FIGS. 6A and 6B, the system 100 preferably couples to a detection chamber (e.g., reaction vessel, detection chamber) from a first side 101a of the system, which can restrict heat dissipation from the first side 101a of the system. Furthermore, the second side 101b of the system 100 is typically used for optical imaging for monitoring (e.g., realtime monitoring, delayed monitoring), and further limiting heat-sinking from the second side 101b. Thus, it is preferable for the set of heat sink supports 150 to couple to the system 100 from a side of the system 100 that does not physically interfere with optical imaging apparatus interfacing with the system 100. However, alternative configurations of the set of heat sink supports 150 can comprise coupling at any suitable side and/or any number of sides of the system 100.

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As shown in FIGS. 8A and 8B, the set of heat sink supports 150 can be configured in any of a number of variations. In a first variation, each heat sink support 151 can be directly placed against a first surface 105a of heatersensor die 111 opposing that of a second surface 105b 5 contacting a detection chamber, as shown in FIG. 7A. The first variation enables efficient transfer of heat out of the first surface 105a of the heater-sensor die away from a respective detection chamber; however, excessive heat sinking can affect heating ramp rates. In a second variation, the system 10 100 comprises a thermally insulating assembly 152 between a heater-sensor die 111 and a corresponding heat sink support 151, as shown in FIG. 7B. In the second variation, the electronics substrate 140 can serve as the thermally insulating assembly 152 and can be situated between the 15 heater-sensor die 111 and a heat sink support 151. Furthermore, in the second variation, a suitable thermal resistance provided by the electronics substrate 140 (e.g., through thickness, material selection, a combination of features) could produce a thermal couple between the heater-sensor 20 die 111 and the heat sink support 151 to permit the heating capacity of the heater-sensor die 111 to achieve the heating times and/or heating ramp rate required by the application, while still allowing adequate cooling rates. Additionally, the second variation can provide increased backside support to 25 each of the set of heater-sensor dies 110 as well as increased surface for adhesion.

In specific examples of the second variation, heat sinking and supporting the "backside" of the electronics substrate 140 can be implemented across multiple heater-sensor dies 30 in, separated by Society for Laboratory Automation and Screening (SLAS) standard spacings, such as 9 mm, 4.5 mm or 2.25 mm spacings. The heat sink support 151 material (e.g., aluminum, copper, silver) in the specific examples is mated with the electronics substrate 140 at each heater- 35 sensor die location, with an air gap positioned laterally between each heater-sensor die location. This configuration can further function to reduce cross talk across a set of detection chambers in contact with the set of heater-sensor dies no. The set of heat sink supports 150 can, however, be 40 configured in any other suitable manner to provide heat dissipation within the system 100, without obstruction of optical detection apparatus, and with provision of desired heat ramping and/or cycling behavior.

In specific examples of the second variation, heat sinking 45 and supporting the backside (i.e., first side 141) of the electronics substrate 140 can be implemented across multiple heater-sensor dies in, separated by Society for Laboratory Automation and Screening (SLAS) standard spacings, such as 9 mm, 4.5 mm or 2.25 mm spacings. The heat sink 50 support 151 material (e.g., aluminum, copper, silver) in the specific examples is mated with the electronics substrate 140 at each heater-sensor die location, with an air gap between locations. This configuration can further function to reduce cross talk between at least a first detection chamber and a 55 second detection chamber interfacing with the system 100.

The set of elastic elements 160 is preferably coupled to a first surface 104a of the electronics substrate 140, and functions to promote contact between the set of heatersensor dies 110 and detection chambers (e.g., reaction vessels, detection chambers) for sample processing according to molecular diagnostic protocols. The set of elastic elements 160 can comprise any one more of springs and elastomeric elements, which can deform and provide transmit a biasing force, through the electronics substrate 140, to reinforce 65 contact between a set of detection chambers and the set of heater-sensor dies 110. The set of elastic elements 160 can,

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however, additionally or alternatively include any other suitable elements configured to provide a biasing force that reinforces contact between a set of detection chambers and the set of heater-sensor dies 110 in an elastic or a non-elastic manner. In one such alternative variation, the system 100 can include one or more actuators configured to drive each of the set of heater-sensor dies in toward a corresponding detection chamber, and in another such alternative variation, the system 100 can include a set of magnets (e.g., including magnet pairs surrounding the set of heater-sensor dies no and a corresponding set of detection chambers), that function to reinforce coupling between the set of heater-sensor dies 110 and the set of detection chambers. However, any other suitable elements can additionally or alternatively be used to facilitate uniform and consistent coupling between the set of heater-sensor dies 110 and a set of detection chambers.

In embodiments of the system 100 including a set of elastic elements 160, the set of elastic elements 160 is preferably coupled to a first surface 104a of the electronics substrate 140, as shown in FIG. 8C, such that each elastic element in the set of elastic elements 160 facilitates contact between a heater-sensor die 111 and a corresponding detection chamber. In a first variation, the set of elastic elements 160 is coupled to first surface 104a of a flexible PCB of the electronics substrate 140, as shown in FIG. 5A. In the first variation, contact between each heater-sensor die 111 and a corresponding detection chamber is thus maintained by a biasing force provided by an individual spring through the flexible PCB of the electronics substrate 140. In the first variation, the number of elastic elements in the set of elastic elements 160 is equal to the number of heater-sensor dies in the set of heater-sensor dies 110, such that the set of elastic elements 160 and the set of heater-sensor dies 110 are paired in a one-to-one manner. Alternatives to the first variation can, however, comprise any suitable number of elastic elements in relation to a number of heater-sensor dies 110. In a second variation, the set of heater-sensor dies 110 is coupled to a second surface 104b of a rigid PCB of the electronics substrate 140, with the set of elastic elements 160 coupled to the first surface 104a of the rigid PCB. In the second variation, the set of elastic elements 160 thus functions to collectively transfer a force through the rigid PCB to maintain contact between the set of heater-sensor dies 110 and the detection chambers. Alternatives to the second variation can also comprise any suitable number of springs in relation to a number of heater-sensor dies in the set of heater-sensor dies 110. Furthermore, variations of the system 100 can include one or more elastic elements coupled to any other elements directly or indirectly coupled to the set of heater-sensor dies 110. For instance, the system 100 can additionally or alternatively include one or more springs 160' coupled to base surfaces of the set of heat-sink supports 150 interfacing with the set of heater-sensor dies, in order to transmit biasing forces.

As shown in FIG. 1, the system 100 can further comprise a controller 165, which functions to automate and/or control heating parameters provided by the set of heater-sensor dies no. The controller 165 can further be configured to provide heat parameter output commands to the heating element(s) 114, and/or configured to receive communication of heating parameters (e.g., detected temperatures) sensed at the sensing element(s) 115 of the system 100. The controller 165 preferably comprises a proportion-integral-derivative (PID) controller, but can alternatively be any other suitable controller 165. The controller 165 preferably interfaces with the set of heater-sensor dies no through the electronics substrate

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140 by a connector; however, the controller 165 can interface with the set of heater-sensor dies no in any alternative suitable manner. Preferably, the controller 165 is configured to automate and control heat output parameters, including any one or more of: heating temperatures, heating ramp rates, heating times (e.g., holding times), and any other suitable heating parameter(s). Furthermore, the controller 165 can be configured to control individual heater-sensor dies 111 in order to provide unique heating parameters for individual detection chambers and/or can be configured to provide common heating parameters for all heater-sensor dies 111 in the set of heater-sensor dies no. In a specific example, the controller 165 comprises a Yokogawa UT750 PID controller, an Arduino UNO R3 microcontroller configured to cycle the UT750 through temperature stages and to control temperature holding, a resistance-to-voltage conversion circuit, and two power supplies—a first power supply configured to supply power to the set of heater-sensor dies 110 and a second power supply configured to supply 20 voltage to the resistance-to-voltage conversion circuit. In the specific example, the controller 165 comprises a resistanceto-voltage conversion circuit because the UT750 PID controller requires voltage as an input for PID control. In another specific example, the controller 165 comprises a 25 National Instruments LabView based system comprised of an NI cDAQ-9178 chassis with an NI 9219 universal analog input card and an NI 9485 eight-channel solid-state relay sourcing or sinking digital output module solid-state relay card. In this specific example, the cDAQ-9178 supports the 30 NI 9219 and NI 9485 cards, the NI 9219 is used to obtain the RTD inputs, and the NI 9485 cycles the power supply voltage to individual heater-sensor dies of the set of heatersensor dies 110. Further, in this specific example, the controller 165 is expandable to 12 or more channels through the 35 use of additional NI 9219 and NI 9485 cards, each of which can handle several channels.

As shown in FIGS. 9A and 9B, the system 100 can further comprise a cooling subsystem 170, which functions to provide heat transfer from the system 100 in order to further 40 enhance controlled heating and cooling by the system 100. The cooling subsystem 170 is preferably configured to provide at least one of convective cooling and conductive cooling of the system 100, but can alternatively be configured to provide any other suitable cooling mechanism or 45 combination of cooling mechanisms. In one variation, the cooling subsystem 170 can comprise a fan 171 that provides convective heat transfer from the system 100. In this variation, the fan 171 can be coupled to any suitable element of the system 100, such as the set of heat sink supports 150, as 50 shown in FIG. 9A. Furthermore, alternatives to this variation can comprise any suitable number of fans of any suitable dimension and configuration, examples of which are shown in FIGS. 9A and 9B. In one such example, the system can include a set of cooling elements integrated with each heat 55 sink support of the set of heat sink supports. In another variation, the cooling subsystem 170 can additionally or alternatively comprise a Peltier device, as shown in FIG. 9C. The Peltier device can be cooled and maintained at a defined temperature (e.g., in the 10-25 C range) to provide a 60 substantial temperature gradient for cooling during a thermal cycling process, which can decrease cooling times and/or cycle times. In yet another variation, the cooling subsystem 170 can additionally or alternatively comprise a liquid cooling system (e.g., water cooling system) configured to 65 surround and absorb heat from one or more heater-sensor dies of the set of heater-sensor dies no, for instance, by way

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of the set of heat sink supports 150. The cooling subsystem 170 can additionally or alternatively comprise any other suitable cooling element(s).

In some variations, reflection from the set of heater-sensor dies no can interfere with light transmitted to photodetectors of an optical subsystem opposed (e.g., directly opposed, in opposition) to the set of heater-sensor dies no (e.g., light emitted from the set of biological samples, light transmitted through filters of an optical subsystem), especially in configurations wherein a set of detection chambers is configured between the set of heater-sensor dies and optical elements of an optical subsystem. In these variations, the set of heatersensor dies 110 can include elements that reduce or eliminate reflection from any portion of the set of heater-sensor dies (e.g., reflection from the heating region, etc.), thereby facilitating analysis of a set of biological samples within the set of detection chambers. In one variation, the set of heater-sensor dies 110 can include or be coupled to one or more non-reflective coatings 180 at surfaces of the set of heater-sensor dies 110 upon which light from the optical subsystem impinges. In a specific example, the non-reflective coating 180 can comprise a high-temperature paint (e.g., dark paint, flat paint) that functions to absorb and/or diffuse light from an opposing optical subsystem, while facilitating heat transfer to a set of detection chambers in thermal communication with the set of heater-sensor dies no. In another variation, the set of heater-sensor dies no can be configured to be in misalignment with photodetectors of the optical subsystem, such that reflection does not interfere with light transmitted to the photodetectors of the optical subsystem. In one example, the set of heater-sensor dies no can be configured to heat a set of detection chambers from a first direction, and the optical subsystem can be configured to receive light from the set of detection chambers from a second direction (e.g., a direction non-parallel to the first direction), such that reflection from the set of heater-sensor dies no does not cause interference. In still other variations, the set of heater-sensor dies no can include any other suitable elements (e.g., coatings, layers, etc.) and/or be configured in any other suitable manner that eliminates, prevents, or mitigates reflection from the set of heater-sensor dies 110 from interfering with light transmitted to photodetectors of an optical subsystem in opposition to the set of heater-sensor dies 110.

Variations of the system 100 can, however, include any other suitable element(s) configured to provide uniform, accurate, precise, and reliable heating of one or more detection chambers in thermal communication with the system 100. Furthermore, as a person skilled in the art will recognize from the previous detailed description and from the figures, modifications and changes can be made to the preferred embodiments of the system 100 without departing from the scope of the system 100.

#### 2. Method of Assembly

As shown in FIG. 10, a method 200 of assembling an embodiment of a thermocycling system 100 comprises forming a first insulating layer coupled to exposed surfaces of a substrate S210, depositing a heating region upon the first insulating layer S220, producing a heating pattern within the heating region, thus producing a heating array S230, dividing the heating array into a set of heater-sensor dies S240, and coupling the set of heater-sensor dies to a electronics substrate S250. The method 200 can further comprise forming a second insulating layer upon the heating region S260, which functions to electrically isolate the heating region on a first side and a second side. The method 200 functions to produce a thermocycling system 100,

embodiments, variations, and examples of which are described above, wherein the thermocycling system 100 provides rapid and uniform thermocycling of samples and

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provides rapid and uniform thermocycling of samples and comprises elements configured to prevent mechanical failure

Block S210 recites: forming a first insulating layer coupled to exposed surfaces of a substrate, and functions to generate a layer that electrically insulates the heating region deposited in Step S220. The substrate is preferably a silicon substrate, but can alternatively be any other suitable semiconducting, or non-conducting substrate. As such, in variations, the substrate can be composed of a semi-conducting material (e.g., silicon, quartz, gallium arsenide), and/or an insulating material (e.g., glass, ceramic). In some variations, the substrate 130 can even comprise a combination of 15 materials (e.g., as in a composite, as in an alloy). In examples wherein the substrate is a silicon substrate, the substrate can be composed of silicon with any suitable type (e.g., P-type), doping (e.g., boron-doping), miller index orientation, resistivity, thickness, total thickness variation, 20 and/or polish.

In forming the first insulating layer, Block S210 can be performed using any one or more of: thermal oxide growth, chemical vapor deposition (CVD), spin coating, spray coating, and any other suitable method of depositing a localized 25 layer of an insulating material. Preferably, the first insulating layer is composed of an insulating oxide material, and in examples can include any one or more of: a thermally grown silicon oxide, a chemical vapor deposited oxide, a deposited titanium oxide, a deposited tantalum oxide, and any other 30 suitable oxide grown and/or deposited in any other suitable manner. However, the first insulating layer can additionally or alternatively include an insulating polymer (e.g., a polyimide, a cyanate ester, a bismaleimide, a benzoxazine, a phthalonitrile, a phenolic, etc.) that is chemical and heat 35 resistant and/or any other suitable material (e.g., chemical vapor deposited nitride, other nitride, paralene, etc.) that is configured to provide the first insulating layer.

In one example of Block S210, the first insulating layer comprises an oxide material, and is formed by growing the 40 oxide material on a substrate. In one example of Block S210, the insulating layer comprises a 0.2 mm layer of silicon oxide, and is formed on a 100 mm silicon wafer using thermal oxidation at 900° C. using water vapor (i.e., in wet oxidation) or oxygen (i.e., in dry oxidation) as the oxidant. 45 In alternative variations and examples of Block S210, the first insulating layer can be formed using high or low temperature thermal oxidation, using any suitable oxidant, and/or using any other suitable method (e.g., fluid deposition of an electrically insulating polymer, softbaking/hardbaking 50 of a deposited polymer, etc.).

Block S220 recites depositing a heating region upon the insulating layer, and functions to form a thermally conductive substrate that is robust during rapid thermocycling. Preferably, the heating region comprises a metal or a metal 55 alloy and can comprise multiple layers; however, the heating region can alternatively comprise any suitable thermally conducting material, and can comprise any suitable number of layers. Additionally, the heating region is preferably deposited in a uniform layer; however, the heating region 60 can be deposited non-uniformly in other variations. In one variation, the heating region comprises an adhesion material layer and a noble material layer, wherein the noble material layer is deposited upon the adhesion material layer after the adhesion material layer is deposited upon the first insulating 65 layer. In examples of this variation, the adhesion layer can comprise chromium or titanium, and the noble layer can

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comprise gold or platinum. In one example of Block S220, the conductive material(s) of the heating region is(are) deposited using an evaporation process; however, in other examples, the conductive material(s) can be deposited by sputtering, plating (e.g., chemical plating, electrochemical plating), or any other suitable method (e.g., electrodeposition). Furthermore, in examples wherein a heating region material is evaporated or sputtered, the insulating layer-substrate subassembly generated in Block S210 can be translated or rotated in order to facilitate uniform deposition of the heating region material.

Block S230 recites producing a heating pattern within the heating region, and functions to produce a heating array characterized by a heating pattern that provides uniform heating and desirable resistances for heating elements and/or sensing elements (e.g., RTDs) defined within the heating region. As such, Block S240 preferably produces a heating pattern having geometric features (e.g., width, thickness, length, spacing) that facilitate uniform heating and provide desired heating and sensing characteristics (e.g., resistance characteristics). In some variations, the pattern can define any one or more of: linear segments, non-linear segments, boustrophedonic segments, continuous segments, non-continuous segments, and any other suitable segment(s) having any other suitable geometry (i.e., width, depth, height, length, path, etc.) In a specific example, the heating pattern was designed using a layout editor (e.g., Mentor Graphics™ or L-Edit<sup>TM</sup>), and comprises a boustrophedonic pattern that is coarse for heating elements and fine for sensing elements. In alternative variations, the heating pattern can be designed using any other suitable method, and can alternatively or additionally comprise any features that contribute to uniform heating and/or suitable resistance ranges. During implementation of Block S230, the heating pattern can be produced photolithographically using a positive resist process, as shown in FIG. 11A. In one example, the heating region can be covered with positive photoresist (e.g., a photomask designed according to the heating pattern) and lithographically etched in exposed regions. In the example, the positive photoresist can then be removed to reveal the heating pattern. In other variations, the heating pattern can be produced using any lithographic method, using positive and/or negative etching to form the heating pattern, and/or using any other suitable method. In one example of an alternative implementation of Step S230', the heating pattern can be produced using a lift-off process, as shown in FIG. 11B, wherein a sacrificial layer is used to define the heating pattern, the heating region material(s) is(are) deposited, and then the sacrificial layer is removed to reveal the heating pattern.

Block S240 recites dividing the heating array into a set of heater-sensor dies, and functions to divide the heating array into a set of heater-sensor dies configured to heat multiple detection chambers (e.g., reaction vessels, sample containers, wells of a plate, chambers of a cartridge) in parallel (e.g., simultaneously, in sequence). Block S240 can also comprise cleaning and drying the heating array prior to and/or after dividing the heating array into a set of heater-sensor dies. Upon completion of Block S240, individual heater-sensor dies of the set of heater-sensor dies can be coupled to one or multiple electronics substrates in order to provide uniform heating of individual sample containers with independent control of heating parameters provided at each of the set of heater-sensor dies. In Block S240, the heating array is preferably divided using a dicing method (e.g., mechanical dicing by saw, laser dicing, water cutting, stealth dicing, etc.), but can additionally or alternatively be divided using

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any other suitable method (e.g., dice before grind). Furthermore, the heating array is preferably divided into rectangular dies, but can alternatively be divided into dies of any suitable morphology (e.g., polygonal dies, non-polygonal dies, circular dies, ellipsoidal dies, etc.). In a specific 5 example, as shown in FIG. 3A, each heater-sensor die produced after division of the heating array has dimensions of approximately 8.6 mm×7 mm, with a circular heating surface that is 5 mm in diameter.

Block S250 recites coupling the set of heater-sensor dies 10 to an electronics substrate, and functions to provide a set of robust connections between the set of heater-sensor dies and an electronics substrate. Coupling in Step S250 comprises forming an electrical connection between connection points on the heater-sensor dies and the electronics substrate, which 15 enables driving of a heating current from the electronics substrate to each of the set of heater-sensor dies (e.g., simultaneously, non-simultaneously). The electrical connection can be provided by a conducting wire (e.g., aluminum wire, gold wire, copper wire) of any suitable thickness (e.g., 20 10-200 microns), or by soldering. Furthermore, coupling in Block S250 preferably comprises coupling heater-sensor dies with a suitable center-to-center spacing that accommodates the spacing of detection chambers intended to be heated by the system. In a specific example, coupling in 25 Block S250 comprises providing a center-to-center spacing between heater-sensor dies of 9 mm, 4.5 mm, or 2.25 mm according to Society of Laboratory Automation Standards (e.g., SLAS Microplate Standards).

Preferably, for a heater-sensor die, Block S250 comprises 30 forming a reverse wire bond between a connection point (i.e., pad) on the heater-sensor die and a connection point (i.e., pad) on the electronics substrate, as shown in FIGS. 5A-5B and 6A-6D. The reverse wire bond prevents unbonding of a heater-sensor die from the electronics substrate, 35 which can be caused due to mechanical forces on the wire bond and/or heater-sensor die, or fatigue failure of a connection. In Block S250, the reverse wire bond is preferably made from a back-side of the electronics substrate, in the orientation shown in FIGS. 5A-5B, through an aperture 40 defined within the electronics substrate. The aperture can be a single aperture, or a set of apertures, and furthermore, multiple heater-sensor dies of the set of heater-sensor dies 110 can be configured to couple to the electronics substrate through an aperture defined within the electronics substrate. 45 The mappings between pads on the heater-sensor dies and the electronics substrate can be one-to-one or many-to-one in variations of coupling. Also shown in FIGS. 5A-5B, Block S250 can additionally comprise depositing an adhesive layer comprising cyanoacrylate and/or any other suit- 50 able adhesive material between a heater-sensor die and the electronics substrate.

In a first variation, as shown in FIG. 6A, Block S250 comprises coupling a heater-sensor die to a first side of the electronics substrate by a wire bond that passes through an 55 aperture defined within the electronics substrate, such that the wire bond couples at one end to a second side of the electronics substrate. In this variation, a connection point on a surface of the heater-sensor die closer to the first side of the electronics substrate is coupled to a connection point on the 60 second side of the electronics substrate, by way of the aperture and the reverse wire bond. In the first variation of Block S250, the heater-sensor die can be further stabilized in place by depositing an adhesive layer at the first side of the electronics substrate.

In a second variation, as shown in FIG. 6B, Block S250 comprises coupling a heater-sensor die to a first side of the

electronics substrate by a wire bond that passes into an aperture defined within the electronics substrate. In this variation, the wire bond is configured to couple, at one end, to a surface of the heater-sensor die closer to the first side of the electronics substrate, and configured to terminate at a connection point part-way between a first side and a second side of the electronics substrate, such that the wire bond is not exposed at the second side of the electronics substrate. In the second variation of Block S250, the heater-sensor die 111 can also be stabilized in place by depositing an adhesive layer at the first side of the electronics substrate.

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In a third variation, as shown in FIGS. 6C-6D, Block S250 comprises providing a recess within the electronics substrate at a first side of the electronics substrate, and coupling (e.g., mounting) a heater-sensor die within the recess of the electronics substrate, wherein the recess is connected to (e.g., contiguous with) an aperture defined within the electronics substrate. Providing the recess of the third variation preferably comprises forming the electronics substrate with a recess and aperture contiguous with the recess, wherein examples of forming can include any one or more of: molding (e.g., injection molding), casting, printing (e.g., 3D printing), and any other suitable method of forming the electronics substrate. Providing the recess of the third variation can additionally or alternatively comprise a method of removing material from a substrate, such as etching, machining (e.g., drilling, milling), and any other suitable method of material removal.

As shown in FIG. 6C, a first example of providing the recess of Block S250 can include providing a recess that is configured such that a heating surface of the heater-sensor die is flush with a first surface of the electronics substrate; however, in an example shown in FIG. 6D, providing the recess can include providing a recess that is configured such that a heating surface of the heater-sensor die is not flush with the first surface of the electronics substrate. In the third variation of Block S250, the wire bond is configured to couple, at one end, to a surface of the heater-sensor die partially situated within the electronics substrate, and configured to terminate at a connection point either part-way between the first side and a second side of the electronics substrate (as in the second variation of Block S250), or at a termination point at the second side of the electronics substrate (as in the first variation of Block S250). Thus, the third variation of Block S250 comprises stabilizing a surface of the heater-sensor die within the recess of the electronics substrate to further prevent shearing or other forms of mechanical failure that could compromise a connection between the heater-sensor die and the electronics substrate. In the third variation, the heater-sensor die can also be further stabilized by providing an adhesive layer within the recess.

In other variations, Block S250 can comprise coupling each heater-sensor die to the electronics substrate by any other suitable method. In one such variation of Block S250, coupling can comprise a "top-side" wire bond, in the orientation shown in FIG. 7A. In this variation, Block S250 comprises coupling a thin wire (e.g., 10-300 microns thick) composed of an electrically conductive material (e.g., aluminum, gold, or copper wire) between a connection point on the heater-sensor die and a connection point on the top side electronics substrate (in the orientation shown in FIG. 7A). Furthermore, in this variation, the "top-side" wire bonds are potted, embedded, or encapsulated to protect them from mechanical failure. In another alternative variation, coupling in Block S250 can comprise forming a flip-chip bond, as shown in FIG. 7B. In this variation, Block S250 can include

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providing a volume (e.g., ball) of solder configured between a connection point on a heater-sensor die and a connection point on the electronics substrate. Furthermore, in this variation, Block S250 can additionally comprise depositing a filler material within regions between the electronics substrate and the heater-sensor die not connected by a volume of solder, in order to further stabilize the assembly. In other variations, the coupling can additionally or alternatively comprise any suitable adhesive (e.g., cyanoacrylate adhesive).

Wire bonding in variations of Block S250 can comprise any suitable combination of the above variations, and can additionally or alternatively comprise any suitable encapsulation, embedding, or potting of wire bonds to further prevent failure in the wire bonds. Furthermore, while variations of Block S250 are described for coupling of a set of heater-sensor dies to an electronics substrate, Block S250 can alternatively comprise coupling of a single heater-sensor die to the electronics substrate, in order to produce a single heating surface configured to heat a detection chamber in thermal communication with the heater-sensor die. However, Block S250 can alternatively comprise coupling any suitable number of heater-sensor dies to any suitable number of electronics substrates.

As shown in FIG. 10, the method 200 can further com- 25 prise Block S260, which recites forming a second insulating layer upon the heating region. Block S260 functions to electrically isolate the heating region on a first side and a second side of the heating region, and is preferably performed prior to coupling of the set of heater-sensor dies to 30 the electronics substrate. However, Block S260 can alternatively be performed at any other suitable time relative to other blocks of the method 200. Preferably, Block S260 comprises depositing (e.g., electrodepositing, using CVD) or growing (e.g., by thermal oxidation) an oxide on the 35 heating region, such that the heating region is "sandwiched" between two oxide layers; however, Block S260 can additionally or alternatively comprise depositing any other suitable insulating material by any suitable method at another surface of the heating region. In one variation, Block S260 40 comprises depositing a low temperature oxide by chemical vapor deposition (e.g., plasma-enhanced chemical vapor deposition) to form the second insulating layer, and in other variations, Block S260 can comprise fluid deposition of an insulating material (e.g., inkjet printing or casting of an 45 electrically insulating polymer, softbaking/hardbaking of a deposited polymer, etc.) onto desired portions of the heating region.

In variations of the method 200 comprising Block S260, the method 200 can also further comprise Block S265, 50 which recites removing material of the second insulating layer to produce a set of connection points. Block S265 functions to provide access to the heating region between insulation regions, such that the heating region can be electrically connected to the electronics substrate. The set of 55 connection points can be defined using a material removal method including any one or more of: etching (e.g., lithography, laser etching), machining (e.g., drilling), and any other suitable method of material removal. In one such variation of Block S265, the set of connection points can be 60 defined photolithographically using a positive resist process in a manner similar to that used in a variation of Block S230. In one example of this variation, the second insulating layer can be covered with positive photoresist and lithographically etched in exposed regions. In the example, the positive 65 photoresist can then be removed to reveal the connection points. In other variations, the connection points can be

defined using any lithographic method, using positive and/or negative etching to form the heating pattern, and/or using any other suitable method. Upon definition of the connection points, the second insulating layer can further be etched (e.g., using buffered hydrofluoric acid) as an additional surface treatment. Alternative variations of Block S265 can include additionally or alternatively removing material from the first insulating surface of Block S210 to form any subset of the set of connection points.

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As shown in FIG. 10, the method 200 can further include Block S270, which recites: coupling a non-reflective coating to at least one heater-sensor die of the set of heater-sensor dies. Block S270 functions to process at least a subset of the set of heater-sensor dies 110 so that they do not interfere with light transmitted to photodetectors of an optical subsystem opposed (e.g., directly opposed, in opposition) to the set of heater-sensor dies no (e.g., light emitted from the set of biological samples, light transmitted through filters of an optical subsystem), especially in configurations wherein a set of detection chambers is configured between the set of heater-sensor dies and optical elements of an optical subsystem. The non-reflective coating is preferably coupled identically to all heater-sensor dies of the set of heater sensor dies; however, the non-reflective coating can alternatively be coupled non-identically to one or more heater-sensor dies of the set of heater-sensor dies. As such, in variations, one or more subsets of the set of heater-sensor dies can be coupled to non-reflective coatings in a manner that provides different light reflection properties for the subset(s) of the set of heater-sensor dies.

In Block S270, the non-reflective coating is preferably a material layer that is applied superficial to at least one of the first insulating layer and the second insulating layer processed in variations of Blocks S210 and S260, respectively. In one example, the non-reflective coating processed in Block S270 can comprise a high-temperature paint (e.g., dark paint, flat paint) that functions to absorb and/or diffuse light from an opposing optical subsystem, while facilitating heat transfer to a set of detection chambers in thermal communication with the set of heater-sensor dies. In this example, the high-temperature paint can be applied by any one or more of: brushing, spraying, dipping, printing, and any other suitable method of coupling the high-temperature paint to one or more surfaces of at least a subset of the set of heater-sensor dies. However, the non-reflective coating can alternatively be processed simultaneously with or can comprise one or more of the first insulating layer and the second insulating layer processed in variations of Blocks S210 and S260, respectively. For instance, one or more of the first and the second insulating layer can include a modified oxide layer that has low-reflectivity, thus preventing interference caused by light reflected from the set of heater-sensor dies. In some extreme variations, however, mitigation of interference due to reflected light from the set of heater-sensor dies can be produced by configuring the set of heater-sensor dies to be in misalignment with photodetectors of the optical subsystem, such that reflection does not interfere with light transmitted to the photodetectors of the optical subsystem, in modified versions of Block S270. For instance, the set of heater-sensor dies can be configured to heat a set of detection chambers from a first direction, and the optical subsystem can be configured to receive light from the set of detection chambers from a second direction (e.g., a direction non-parallel to the first direction), such that reflection from the set of heater-sensor dies 110 does not cause interference. In still other variations of Block S270, the set of heater-sensor dies can be processed with any other

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suitable elements (e.g., coatings, layers, etc.) and/or be configured in any other suitable manner that eliminates, prevents, or mitigates reflection from the set of heater-sensor dies from interfering with light transmitted to photodetectors of an optical subsystem in opposition to the set of heater-sensor dies no.

The method 200 can further comprise any other suitable block, such as calibrating sensing elements of the thermocycling system S280. In an example of Block S270, the set of heater-sensor dies coupled to the electronics substrate can 10 be installed in thermal chamber to calibrate the sensing elements (i.e., RTDs) of the set of heater-sensor dies. In the example, the electronics substrate and a first connector end of a calibration system was placed in a thermal chamber and a second connector end of the calibration system was 15 attached to an array of contacts outside the thermal chamber. The thermal chamber was allowed to equilibrate in stages at a series of temperatures spanning the expected dynamic range of the RTDs, from 30 C to 100 C in four stages. The RTD resistance values were read out at the various equili- 20 brated temperatures, and fit a Callendar-Van Dusen equation. The calibration of the example of Block S270 yielded the coefficients used to convert the sensing element resistance values to temperature values, in order to calibrate the sensing elements of the system.

The system 100 and/or method 200 of the preferred embodiment and variations thereof can be embodied and/or implemented at least in part as a machine configured to receive a computer-readable medium storing computer-readable instructions. The instructions are preferably executed 30 by computer-executable components preferably integrated with the system 300 and one or more portions of the processor 350. The computer-readable medium can be stored on any suitable computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices 35 (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or hardware/firmware combination device can alternatively or additionally execute the instruc- 40 tions.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of methods according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the 45 flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can 50 occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the 55 block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and 60 computer instructions.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing 65 from the scope of this invention defined in the following claims.

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We claim:

- 1. A system for thermocycling biological samples within detection chambers comprising:
- a set of heater-sensor dies, each heater-sensor die in the set of heater-sensor dies comprising a heating surface configured to interface with a detection chamber and an inferior surface, inferior to the heating surface, including a connection point, wherein each of the set of heater-sensor dies includes a heating element and a sensing element;
- an electronics substrate, comprising a first substrate surface coupled to the inferior surface of each of the set of heater-sensor dies, a set of apertures longitudinally spaced across the electronics substrate and providing access through the electronics substrate to the set of heater-sensor dies, and a second substrate surface inferior to the first substrate surface,
  - wherein the electronics substrate comprises a set of substrate connection points at least at one of the first substrate surface, an aperture surface defined within at least one of the set of apertures, and the second substrate surface, and wherein the electronics substrate couples the heating element and the sensing element of each of the set of heater-sensor dies to a controller;
- a set of heat-sink supports coupled to at least one of 1) the set of heater-sensor dies, through the set of apertures, and 2) the second substrate surface of the electronics substrate and configured to dissipate heat generated by the set of heater-sensor dies,
  - wherein at least one of the set of heat-sink supports includes an integrated cooling element, and wherein a base surface of each of the set of heat-sink supports is coupled to an elastic element that transmits a biasing force through the electronics substrate, thereby maintaining thermal communication between the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers; and
- a set of wire bonds, including a wire bond coupled between the connection point of at least one of the set of heater-sensor dies and one of the set of substrate connection points.
- 2. The system of claim 1, wherein the set of substrate connection points is distributed at regions of the second substrate surface between adjacent apertures of the set of apertures of the electronics substrate.
- 3. The system of claim 1, wherein the electronics substrate further includes a set of recesses, each recess of the set of recesses contiguous with at least one aperture of the set of apertures.
- **4**. The system of claim **3**, wherein each recess of the set of recesses is configured to receive a heater-sensor die of the set of heater-sensor dies.
- 5. The system of claim 3, wherein each heater sensor-die of the set of heater-sensor dies is coupled to the first substrate surface, and the set of substrate connection points is defined between the first substrate surface and the second substrate surface within the set of recesses.
- 6. The system of claim 1, wherein the electronics substrate is a flexible electronics substrate, and wherein the system further comprises a set of elastic elements coupled to the second substrate surface of the flexible electronics substrate and configured to transmit a set of biasing forces through the flexible electronics substrate and to the set of heater-sensor dies, thereby maintaining thermal communication between

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es and a set of detection chambers

the set of heater-sensor dies and a set of detection chambers upon alignment of the set of heater-sensor dies with the set of detection chambers.

7. The system of claim 1, wherein each of the set of heater-sensor dies includes a coating, proximal the heating 5 surface, configured to mitigate reflection of light from the heating surface toward photodetectors of an optical subsystem, in a configuration wherein the set of heater-sensor dies is opposed to photodetectors of the optical subsystem.

\* \* \* \* \* 10

# EXHIBIT 30



# (12) United States Patent

Williams et al.

## (10) Patent No.: US 9,604,213 B2

(45) **Date of Patent:** Mar. 28, 2017

#### (54) SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

(71) Applicant: **NeuMoDx Molecular, Inc.**, Ann Arbor, MI (US)

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MI (US)

(\*) Notice: Subject to any disclaimer, the term of this

patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: 14/613,616

(22) Filed: **Feb. 4, 2015** 

(65) Prior Publication Data

US 2015/0151300 A1 Jun. 4, 2015

#### Related U.S. Application Data

(63) Continuation-in-part of application No. 13/766,359, filed on Feb. 13, 2013, now Pat. No. 9,050,594.

(Continued)

(51) Int. Cl.

B01L 3/00 (2006.01)

B01L 7/00 (2006.01)

G01N 35/00 (2006.01)

(52) U.S. Cl.

CPC ..... **B01L** 3/502761 (2013.01); **B01L** 3/50851 (2013.01); **B01L** 3/502738 (2013.01);

(Continued)

(58) Field of Classification Search

CPC ..... B01L 2200/0668; B01L 2200/0684; B01L 2200/0689; B01L 2200/10; B01L 2300/044:

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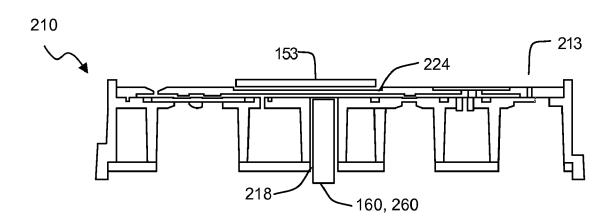
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Primary Examiner — Nathan Bowers
Assistant Examiner — Lydia Edwards
(74) Attorney, Agent, or Firm — Jeffrey Schox; Ivan
Wong

#### (57) ABSTRACT

A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.

#### 14 Claims, 25 Drawing Sheets



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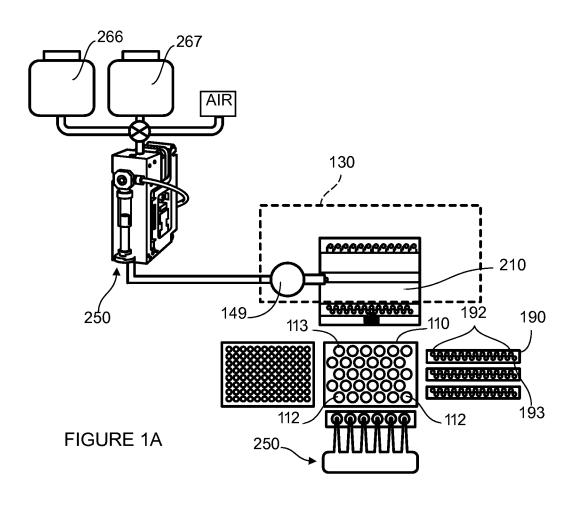
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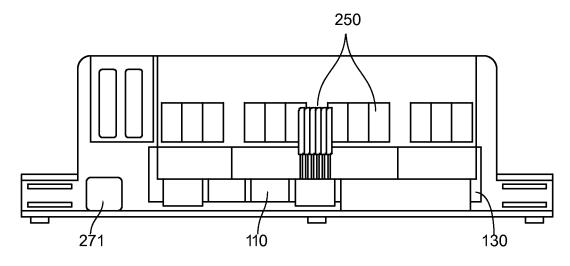


FIGURE 1B

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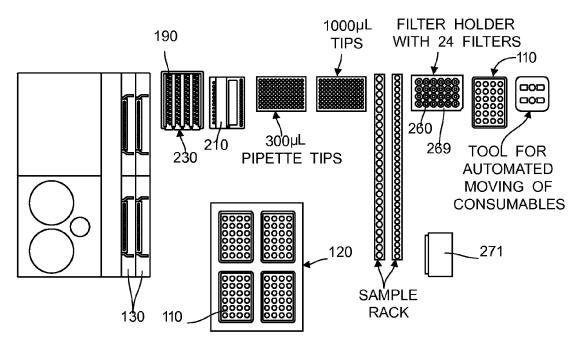


FIGURE 2A

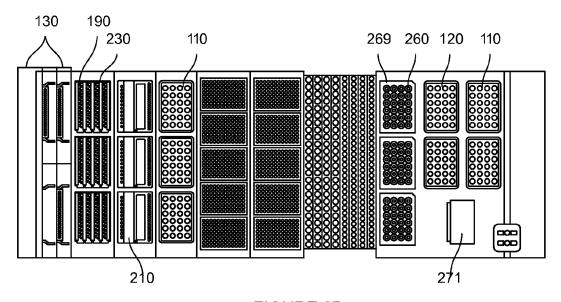


FIGURE 2B

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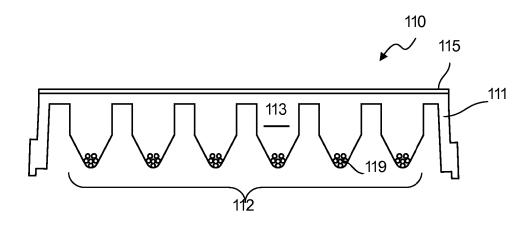


FIGURE 3A

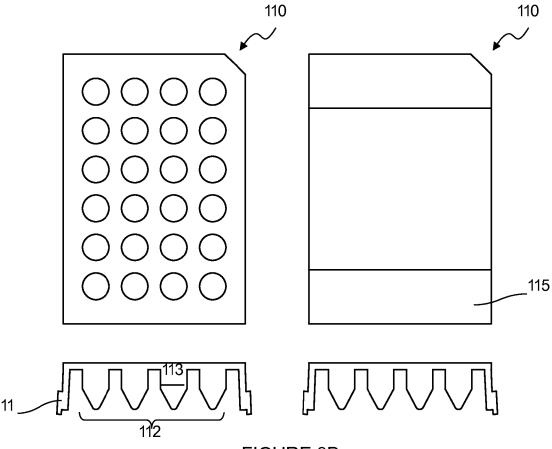
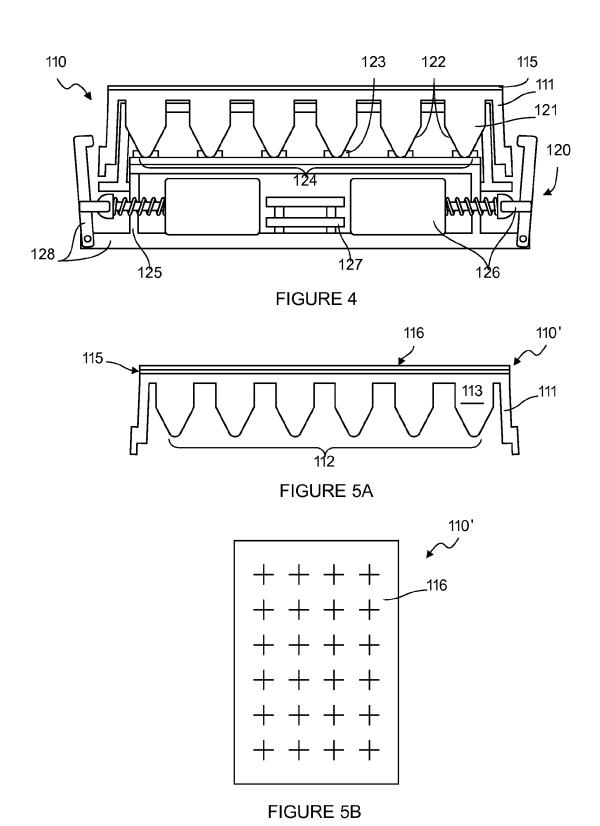


FIGURE 3B

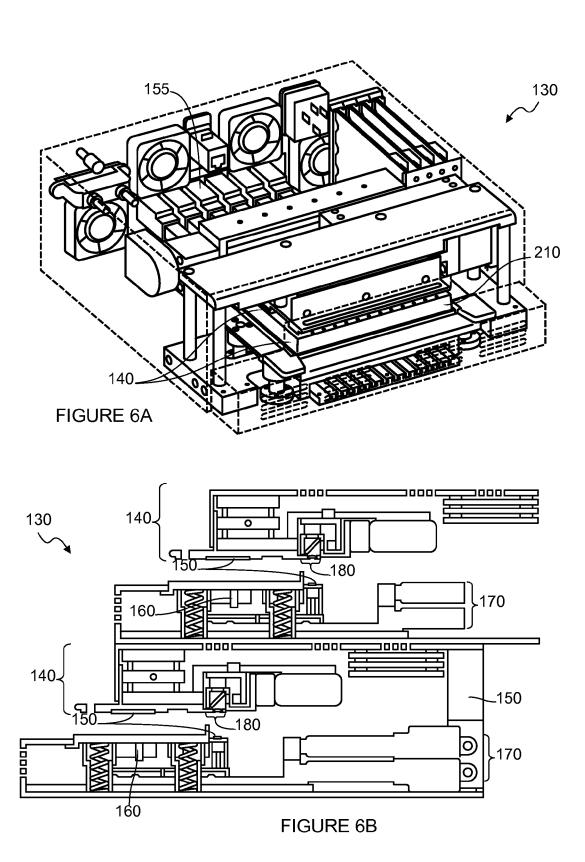
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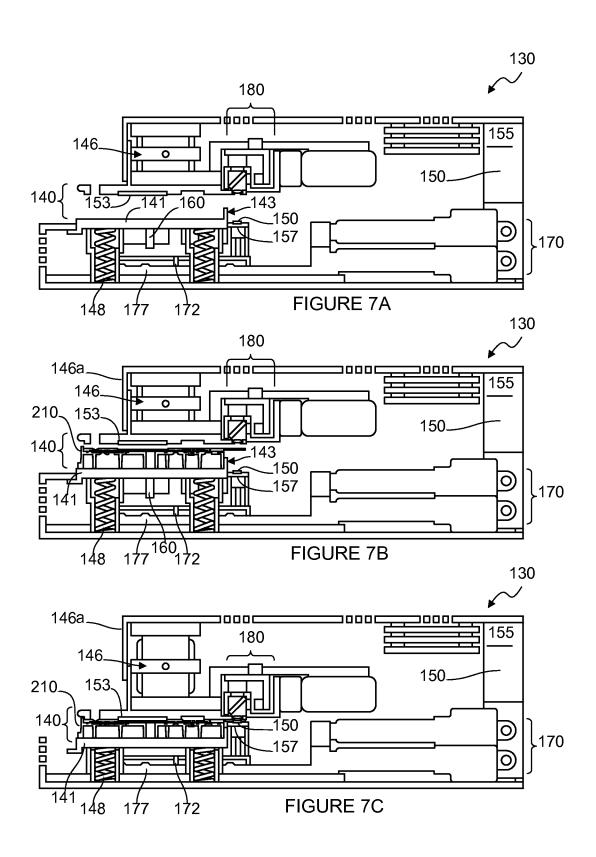
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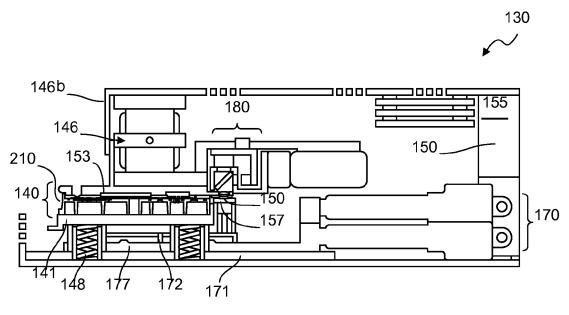


FIGURE 7D

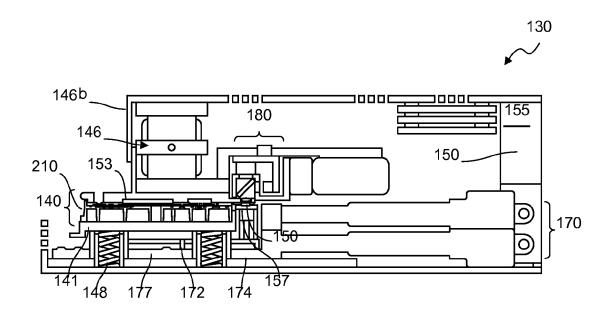
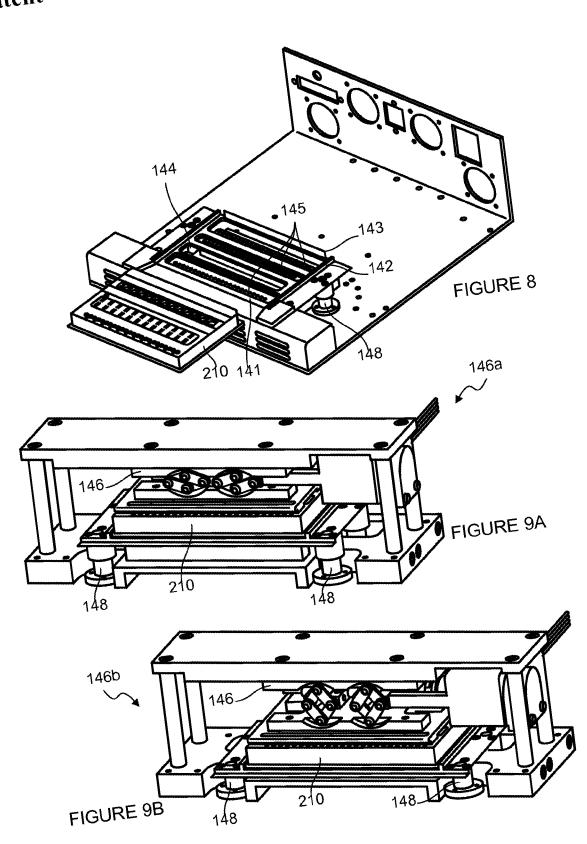
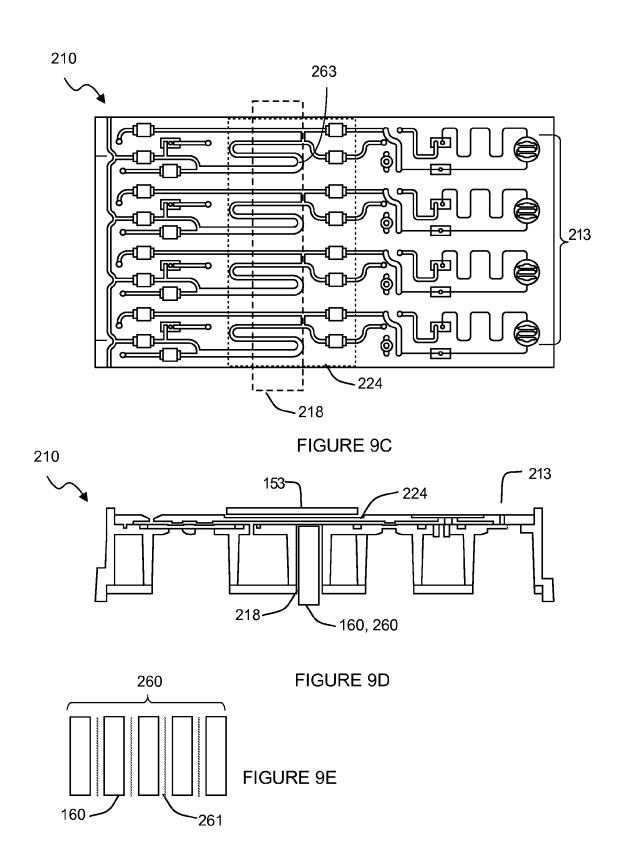


FIGURE 7E

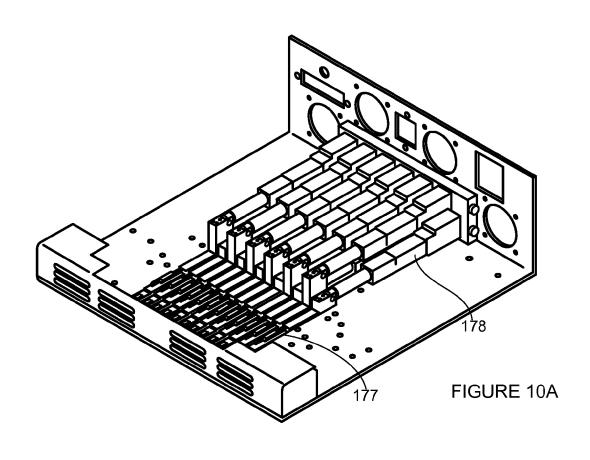


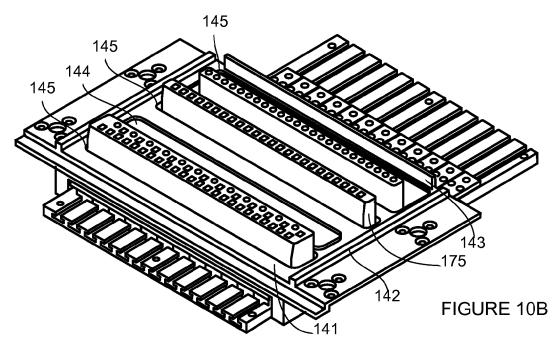
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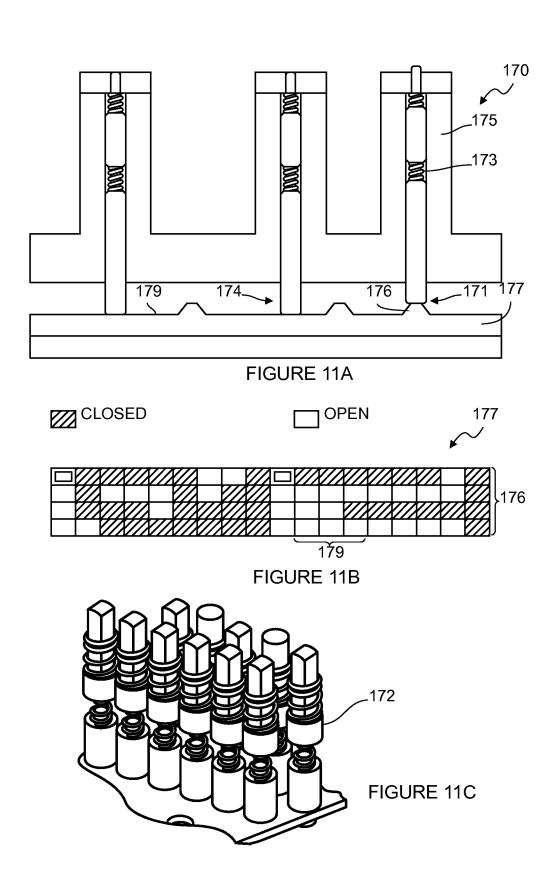
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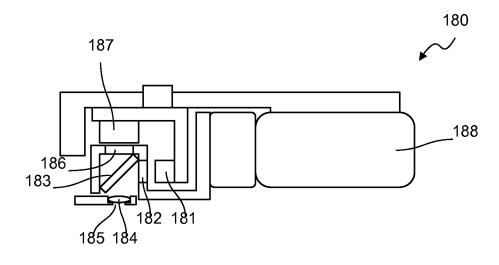


FIGURE 12A

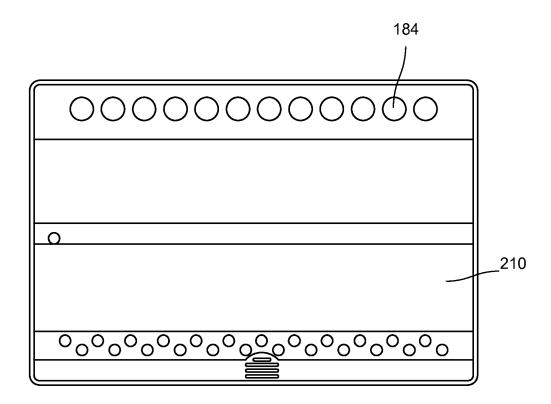
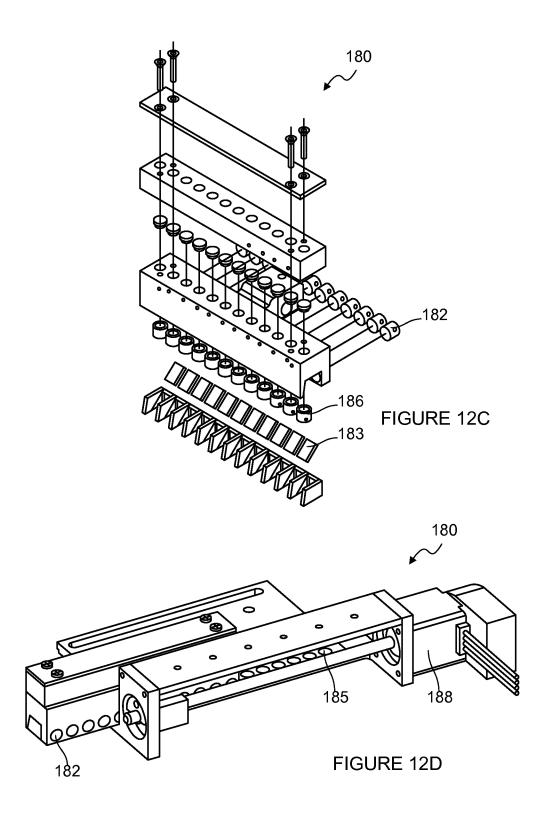


FIGURE 12B

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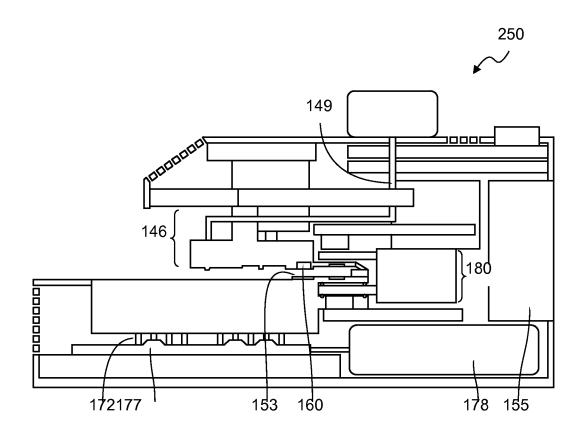
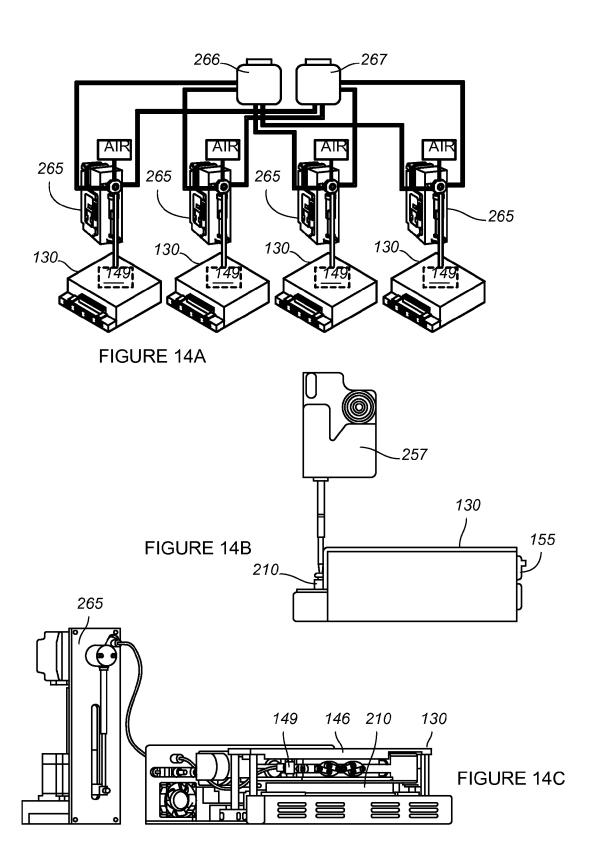


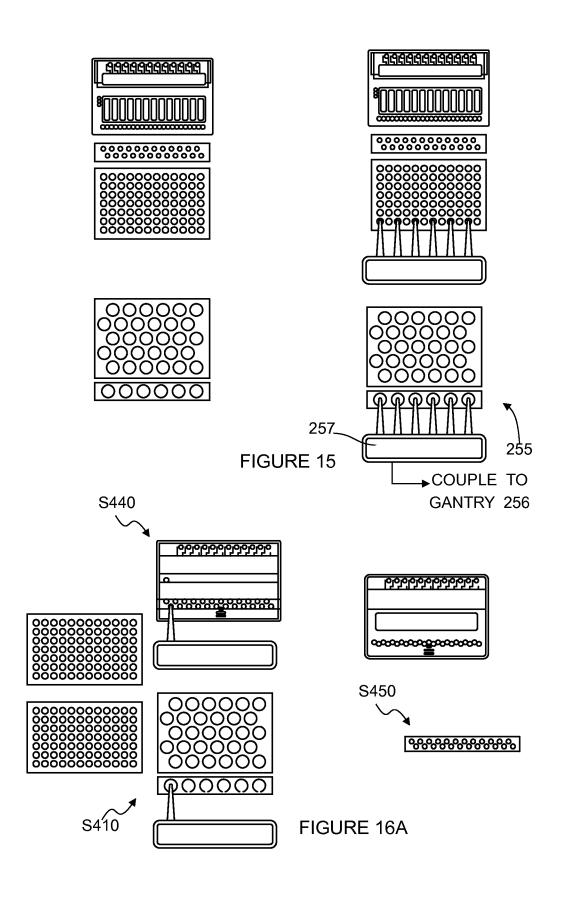
FIGURE 13

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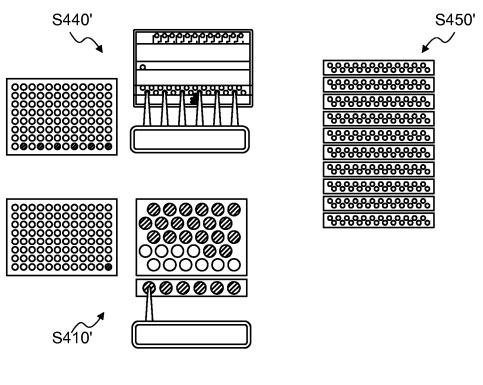
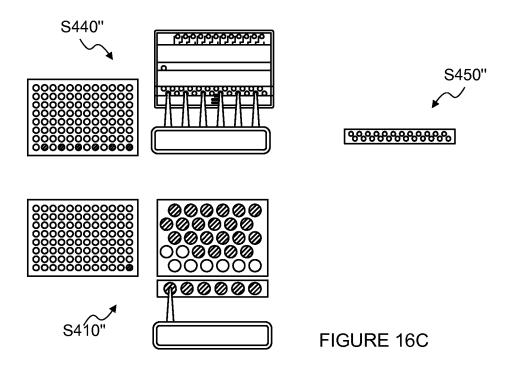


FIGURE 16B



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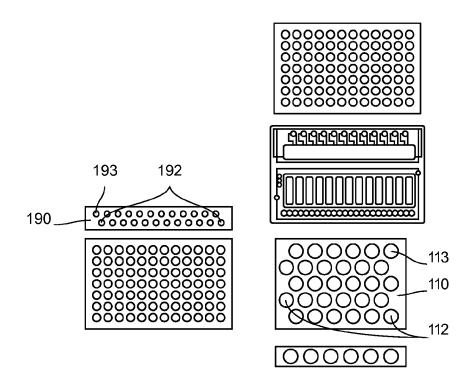


FIGURE 17A

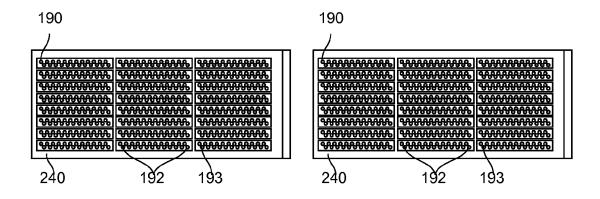


FIGURE 17B

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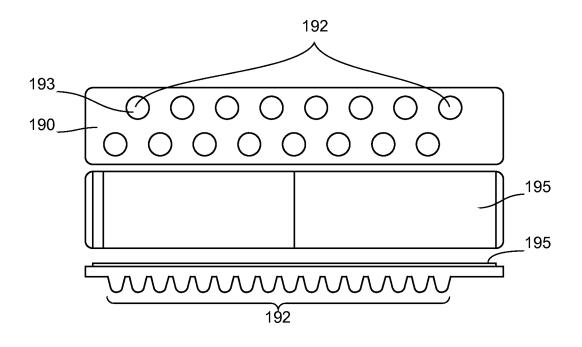
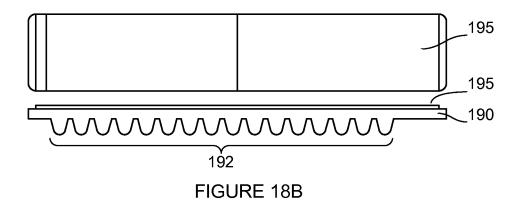
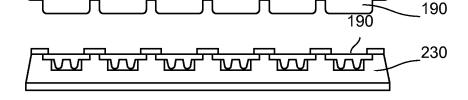


FIGURE 18A



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FIGURE 19

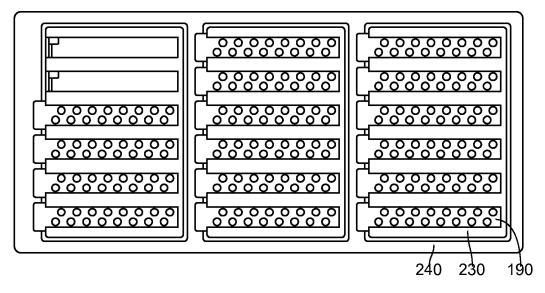


FIGURE 20

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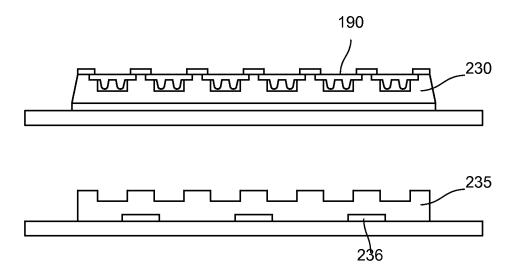


FIGURE 21A

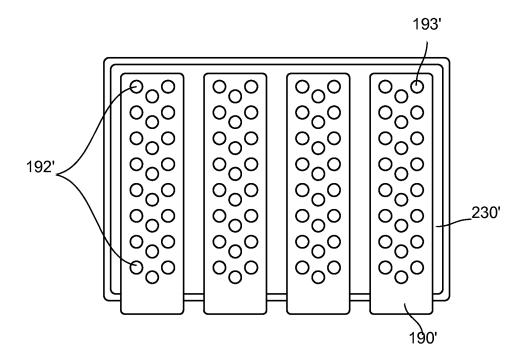
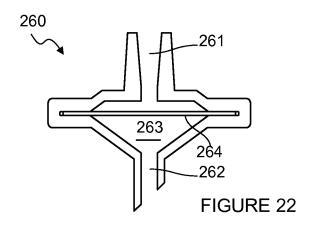
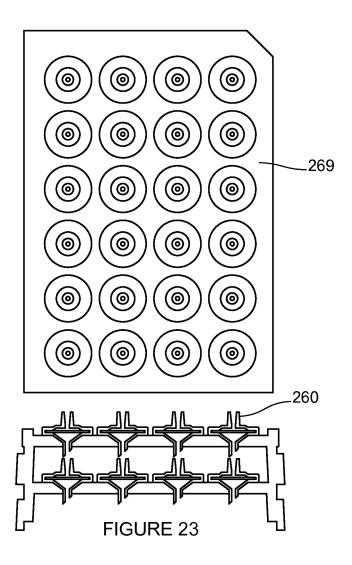


FIGURE 21B

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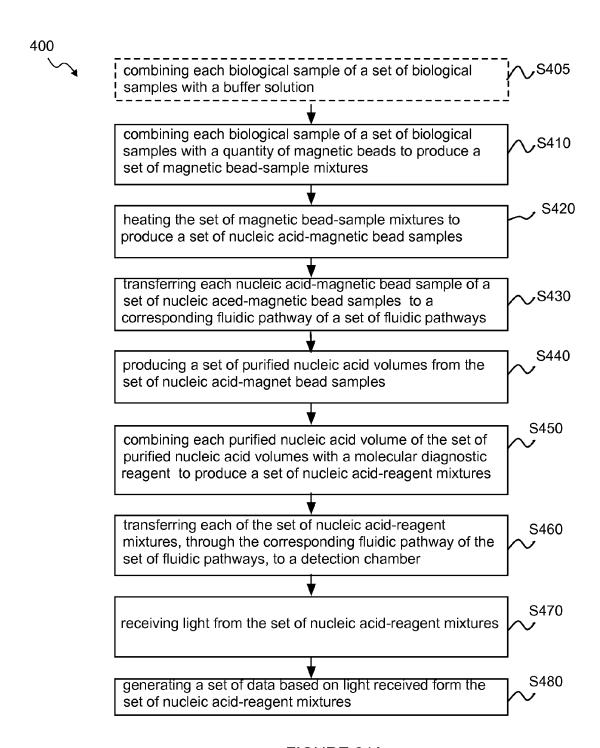


FIGURE 24A

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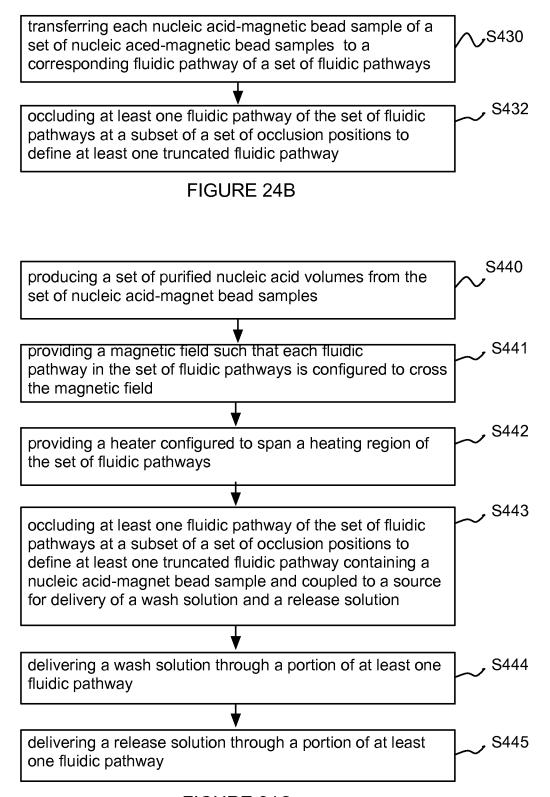


FIGURE 24C

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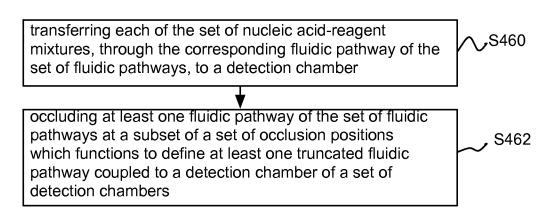


FIGURE 24D

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# SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

# CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of U.S. application Ser. No. 13/766,359 filed on 13 Feb. 2013, which claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are incorporated herein in their entirety by this reference. This application is also related to U.S. application Ser. No. 13/765,996, which is incorporated herein in its entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved system and <sup>20</sup> method for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a clinical laboratory discipline 25 that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic 30 acid (RNA) for diagnostic use in healthcare and other fields involving analysis of nucleic acids. Molecular diagnostic analysis of biological samples can include the detection of one or more nucleic acid materials present in the specimen. The particular analysis performed may be qualitative and/or 35 quantitative. Methods of analysis typically involve isolation, purification, and amplification of nucleic acid materials, and polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, 40 and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecular diagnostic techniques are often labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, process- 45 ing, and amplification are specific to certain sample matrices and/or nucleic acid types and not applicable across common sample and nucleic acid types.

Due to these and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for an 50 improved system and method for processing and detecting nucleic acids. This invention provides such a system and method.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1B depict an embodiment of a system for processing and detecting nucleic acids;

FIGS. 2A-2B depict an embodiment of elements, and a top view of an embodiment of a system worktable, respectively, of an embodiment of a system for processing and detecting nucleic acids;

FIGS. 3A-3B depict an embodiment of a capture plate for combining a sample with magnetic beads;

FIG. 4 depicts an embodiment of a capture plate module 65 to facilitate lysis of a biological sample and combination of the biological sample with magnetic beads;

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FIGS. 5A-5B depict an alternative embodiment of a capture plate;

FIGS. 6A-6B depict embodiments of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 7A-7E depict a sequence of operations performed by elements of an embodiment of a molecular diagnostic module;

FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform;

FIGS. 9A-9B depict configurations of a linear actuator of an embodiment of a molecular diagnostic module;

FIGS. 9C-9E depict configurations of a portion of an embodiment of a microfluidic cartridge and a molecular diagnostic module;

FIGS. 10A-10B depict elements of an embodiment of a valve actuation subsystem of a molecular diagnostic module:

FIGS. 11A-11C depict an embodiment of a valve actuation subsystem of a molecular diagnostic module;

FIGS. 12A-12D depict elements of an embodiment of an optical subsystem of a molecular diagnostic module;

FIG. 13 depicts a side view of an alternative embodiment of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 14A-14C depict an embodiment of a fluid handling system of a system for processing and detecting nucleic acids:

FIG. 15 depicts embodiments of elements of the fluid handling system;

FIGS. 16A-16C are schematics depicting example methods for processing and detecting nucleic acids;

FIGS. 17A-17B show embodiments of consumables and reagents used in a system for processing and detecting nucleic acids;

FIGS. 18A-18B depict an embodiment of an assay strip to facilitate analysis of a sample containing nucleic acids;

FIG. 19 depicts an embodiment of an assay strip holder; FIG. 20 depicts an embodiment of an assay strip carrier; FIGS. 21A-21B show alternative embodiments of assay strip holders and assay strips, respectively;

FIG. 22 shows an embodiment of a filter to facilitate processing and detecting of nucleic acids;

FIG. 23 shows an embodiment of a filter holder to facilitate processing and detecting of nucleic acids; and

FIGS. **24**A-**24**D depict embodiments of a method for processing and detecting nucleic acids.

# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

55 1. System for Processing and Detecting Nucleic Acids

As shown in FIGS. 1A-1B and 7A, an embodiment of a system 100 for processing and detecting nucleic acids comprises: a capture plate 110 configured to facilitate binding of nucleic acids within a biological sample to a set of magnetic beads 119; a molecular diagnostic module 130 comprising a microfluidic cartridge receiving module 140, heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, an optical subsystem 180; and an assay strip 190 configured to facilitate mixing of molecular diagnostic reagents with a nucleic acid volume. Other embodiments of the system 100 may further comprise at least one of a capture plate module 120 configured to support the capture

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plate 110; a filter 200 and filter holder 205 to facilitate sample preparation; a microfluidic cartridge 210 configured to facilitate sample processing; an assay strip holder 230; an assay strip carrier 240; a liquid handling system 250 configured to facilitate gas and fluid delivery to different ele- 5 ments of the system 100; a processor configured to analyze data resulting from a run of the system 100; and a user interface configured to allow a user to interact with the system 100. The system 100 thus functions to receive biological samples containing nucleic acids (i.e., impure 10 nucleic acid samples), separate nucleic acids from the biological samples, and analyze nucleic acid samples according to at least one molecular diagnostic protocol (e.g., PCR). Preferably, the system 100 is a walkaway system by which a user loads a set of biological samples containing nucleic 15 acids, and receives a set of data resulting from a molecular diagnostic protocol without any further sample manipulation by the user. Alternatively, the system 100 facilitates aspects of sample preparation for a molecular diagnostic protocol, with some sample manipulation performed by the user.

In one example workflow of the system 100, a liquid handling system 250 aspirates a set of biological samples containing nucleic acids (i.e., impure nucleic acid samples), and dispenses the set of biological samples into a capture plate 110 to be lysed and combined with magnetic beads 25 (containing a proprietary affinity coating to bind the nucleic acids to the magnetic beads) by a capture plate module 120. The liquid handling system 250 then aspirates substantially all of each sample of the set of lysed biological samples combined with magnetic beads (i.e., set of magnetic bead- 30 samples) from the capture plate no, and dispenses the set of magnetic bead-samples into a microfluidic cartridge 210, aligned within a cartridge receiving module 140 of a molecular diagnostic module 130, and configured to be manipulated by the molecular diagnostic module 130. A 35 heating and cooling subsystem 150, a magnet 160, and a valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate separation of a set of nucleic acids from the magnetic bead-samples, as the liquid handling system 250 dispenses wash solutions, release solutions, 40 and/or air at appropriate stages. The liquid handling system 250 then aspirates the set of nucleic acids from the microfluidic cartridge 210 contained within the molecular diagnostic module 130, combines the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, 45 and dispenses the set of nucleic acids combined with the set of molecular diagnostic reagents (i.e., set of nucleic acidreagent mixtures) into the microfluidic cartridge 210 within the molecular diagnostic module 130. The detection chamber heaters 157, optical subsystem 180 and valve actuation 50 subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user interface.

As stated, the above workflow is just one example workflow of the system 100, and other workflows of the system 100 and methods of processing and detecting nucleic acid samples are further described in Section 2 below. A detailed description of elements of an embodiment of the system 100 are described in sections 1.1-1.6 below.

1.1 System—Capture Plate and Capture Plate Module

As shown in FIGS. 3A and 3B, the capture plate 110 comprises a capture plate substrate in comprising a set of wells 112 and a puncturable foil seal 115, and functions to facilitate binding of nucleic acids within a biological sample 65 to a set of magnetic beads 119. Preferably, the entire capture plate 110 is configured to be a consumable (i.e., disposable),

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such that each well of the capture plate 110 can only be used once yet the remaining unused wells can be used during additional runs of the system 100. Alternatively, at least a portion of the capture plate 110 is configured to be reusable, such that additional mixing or reagent additions can be performed and portions of the capture plate 110 may be used for multiple runs of the system 100. In one variation of the capture plate 110, the capture plate substrate 111 is reusable, while the puncturable foil seal 115 is disposable and replaced after each run of the system 100.

The capture plate substrate 111 is configured such that the capture plate 110 is capable of resting on a flat surface, can be stacked with another capture plate 110, and also can be manipulated with industry standard instrument components for handling of microtiter plates. The capture plate substrate also functions to define the set of wells 112 and to couple to the puncturable foil seal 115. The capture plate substrate in is preferably composed of a PCR-compatible polymer that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 112 of the capture plate substrate in function to receive at least one biological sample which contain or are suspected of potentially containing nucleic acids, and to facilitate combination of the biological sample with a set of magnetic beads 119. Preferably, the wells 113 are each configured to accommodate not only a biological sample, but also to facilitate mixing of the biological sample with a set of magnetic beads 119 (e.g., using a pipettor, the liquid handling system 250 or other apparatus), which preferably are preloaded in wells 112, or alternatively may be added by an operator. Preferably, the wells are also deeper than they are wide to allow a significant number of wells 112 (e.g., 24) with clinically relevant sample volumes, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wider than they are deep to facilitate larger devices for mixing the biological samples with the magnetic beads 119. Each well 113 of the set of wells 112 also preferably has a conically shaped bottom region, as shown in FIG. 3A, to facilitate complete aspiration of a fluid from a well. Alternatively, each well 113 may not have a conically shaped bottom region. Additionally, in the orientation shown in FIG. 3A, the tops of each well 113 in the set of wells 112 preferably form raised edges protruding from the capture plate substrate 111, in order to facilitate sealing of each well 113 by the puncturable foil seal 115. Alternatively, the tops of each well 113 in the set of wells 112 may not form raised edges protruding from the capture plate substrate in. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. However, the magnetic beads may alternatively be any appropriate magnetic beads (e.g., magnetic, parmagnetic, or superparamagnetic) configured to facilitate biomagnetic separation.

Each quantity of magnetic beads 119 may be accompanied by lysing reagents (e.g. proteinase K) and a sample
process control comprising nucleic acid sequences for DNA
and RNA, which function to lyse biological samples and to
provide a mechanism by which sample process controls may
be later detected to verify processing fidelity and assay
accuracy. The sample process control comprising nucleic
acid sequences for DNA and RNA allows one version of the
capture plate to facilitate assays involving DNA and RNA

detection. Preferably, the quantity of magnetic beads 119,

lysing reagents, and sample process controls is dried within each well to improve shelf life; however, the quantity of magnetic beads 119, lysing reagents, and sample process controls may alternatively be in liquid form.

The puncturable foil seal 115 functions to isolate each well 113 of the set of wells 112, prevent contamination of the contents of each of the set of wells 112, protect the magnetic beads 119 and other reagents stored in wells 112 from degradation, and provide information identifying the capture 10 plate no. The puncturable foil seal 115 preferably seals each well 113 of the capture plate 110, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one variation, the puncturable foil seal 115 also forms a seal 15 around an element that punctures it, and in another variation, the puncturable foil seal 115 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 115 is also preferably labeled with identifying information including at least one of manufac- 20 turer information, capture plate contents, the lot of the contents, an expiry date, and a unique electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal 115 does not extend beyond the footprint of the capture plate no, but alternatively, the 25 cradle and support the capture plate 110, and functions to puncturable foil seal 115 may be any appropriate size and/or include protruding features (e.g., tabs) that facilitate handling of the capture plate.

In one variation, the capture plate 110 may be prepackaged at least with magnetic beads 119, such that each well 30 113 in the set of wells 112 is prepackaged with a set of magnetic beads 119 defined by a specific quantity or concentration of magnetic beads. The set of wells 112 may then be sealed by the puncturable foil seal 115, which is configured to be punctured by an external element that delivers 35 volumes of biological samples to be mixed with the magnetic beads 119. In another variation, the capture plate no may not be prepackaged with magnetic beads 119, but the wells 113 of the capture plate may still be sealed with a puncturable foil seal 115. In this variation, the puncturable 40 foil seal 115 is configured to be punctured by at least one external element, for co-delivery of biological samples and magnetic beads intended to be combined.

A variation of the capture plate 110' may further comprise a slotted rubber membrane 116, as shown in FIGS. 5A and 45 5B, configured to provide access through the puncturable foil seal 115 to the set of wells 112. The slotted rubber membrane 116 thus functions to prevent or reduce splashing, evaporation, and/or aerosolization of contents of the set of wells 112. Preferably, the slotted rubber membrane 116 50 comprises slots that are self-sealing and centered over wells of the set of wells 112, and further does not extend beyond the footprint of the capture plate no. Alternatively, the slots of the slotted rubber membrane 116 may not be self-sealing, and/or the slotted rubber membrane 116 may be any appro- 55 priate size and comprise features that extend beyond the footprint of the capture plate no.

In a specific example, the capture plate 110 comprises 24 wells 113 with an 18 mm center-to-center pitch, each well having a volumetric capacity of 2 mL, and is compliant with 60 Society for Laboratory Automation and Screening (SLAS) standards. Each well 113 of the capture plate no in the specific example is also prepackaged with a specified quantity of magnetic beads 119, and comprises a protruding top edge that is heat sealed to a puncturable foil seal. In addition, 65 each well 113 also contains other reagents beneficial for processing and monitoring the sample, including proteinase

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K and one or more specific nucleic acid stands designed to serve as a process control. The specific example of the capture plate 110 can thus combine two groups of 12 biological samples with magnetic beads. The capture plate 110 in the specific example is produced by injection molding, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier.

An embodiment of the system 100 may further comprise a capture plate module 120, as shown in FIG. 4, which functions to receive, support, and heat a capture plate 110. The capture plate module 120 preferably comprises a thermally conducting substrate 121 configured to cradle a capture plate 110, a capture plate heater 123, a capture plate receiving module 125, and a capture plate electronics module 127. Preferably, the capture plate module 120 functions to facilitate lysis of a biological sample deposited into a well 113 of the capture plate, and to facilitate binding of nucleic acids (i.e., within a lysed biological sample) to a quantity of magnetic beads 119 within a well 113 of the capture plate 110. In a specific example, the capture plate module 120 has dimensions of 108 mm×156 mm×45 mm and is configured to rest on a flat surface.

The thermally conducting substrate 121 is configured to conduct heat to the set of wells 112 of the capture plate 110. Preferably, the thermally conducting substrate 121 is also configured to reversibly couple to the capture plate 110, and comprises a set of indentations 122 that encircle each well 113 in the set of wells 112. In one variation, the indentations 122 completely conform to the external surface of each well 113 of the capture plate 110, but in another variation, the indentations 122 may encircle a portion of each well 113 of the capture plate 110. Additionally, the indentations 122 are preferably thermally conducting in order to conduct heat to the set of wells 112, and portions of the thermally conducting substrate 121 aside from the indentations 122 are composed of non-conducting, rigid material. Alternatively, the entire thermally conducting substrate 121 may be composed of a material that is thermally conducting.

The capture plate heater 123 is preferably coupled to the thermally conducting substrate 121, and functions to transfer heat, through the thermally conducting substrate 121, to a well 113 of the capture plate 110. The capture plate heater 123 preferably conforms to at least a portion of an indentation 122 of the thermally conducting substrate 121, to facilitate heat transfer through the indentation 122 to an individual well 113 of the capture plate 110. In this variation, the capture plate heater 123 is one of a set of capture plate heaters 124, wherein each capture plate heater 123 in the set of capture plate heaters 124 transfers heat to an individual well 113 of the set of wells 112 of the capture plate 110. Alternatively, the capture plate heater 123 may conform to portions of multiple indentations 122 of the thermally conducting substrate 121, such that the capture plate heater 123 is configured to transfer heat to multiple wells 113 of the capture plate 110. Preferably, the capture plate heater 123 is a resistance heater, but alternatively, the capture plate heater 123 may be a Peltier or any appropriate heater configured to transfer heat to the capture plate 110. The capture plate heater 123 may also further couple to a heat sink.

The capture plate receiving module 125 comprises a capture plate actuation system 126 that functions to couple the capture plate module 120 to a capture plate 110. As shown in FIG. 4, the capture plate actuation system 126 comprises a structural support with hinged grips 128 and at least one capture plate module actuator 129. The capture

linear actuator 146.

plate module actuator 129 is preferably a push-type solenoid with a spring return, but may alternatively be any appropriate linear actuator, such as a hydraulic actuator. The structural support with hinged grips 128 preferably couples to the capture plate heater 123 and houses the capture plate module 5 actuator 129, such that, in a first configuration, actuation of the capture plate module actuator 129 outwardly displaces the hinged grips (allowing the capture plate module 120 to receive a capture plate 110), and in a second configuration, actuation of the capture plate module actuator 129 inwardly 10 displaces the hinged grips (allowing the capture plate module 120 to couple to the capture plate 110). The structural support with hinged grips 128 may further comprise a textured and/or high-friction surface configured to grip a capture plate 110, but alternatively may not comprise a 15 textured and/or high-friction surface.

The capture plate electronics module 127 is coupled to the capture plate heater 123 and the capture plate actuation system 126, and functions to enable control of the capture plate heater 123 and the capture plate actuation system 126. 20 Preferably, the capture plate electronics module 127 modulates an output of the capture plate heater 123, in order to controllably heat at least one well 113 of the capture plate 110. Additionally, the capture plate electronics module 127 preferably modulates the capture plate actuation system 126, 25 in order to controllably couple the capture plate module 120 to a capture plate 110. Preferably, the capture plate electronics module 127 is coupled to an external power supply, such that the capture plate module 120 does not include an integrated power supply; however, in alternative embodi- 30 ments, the capture plate electronics module 127 may be coupled to a power supply integrated with the capture plate module 120.

#### 1.2 System—Molecular Diagnostic Module

As shown in FIGS. 6A and 6B, an embodiment of the 35 molecular diagnostic module 130 of the system 100 includes a cartridge receiving module 140, a heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, and an optical subsystem 180, and functions to manipulate a microfluidic cartridge 210 for processing of a biologi- 40 cal sample containing nucleic acids. The molecular diagnostic module 130 is preferably configured to operate in parallel with at least one other molecular diagnostic module 130, such that multiple microfluidic cartridges 210 containing biological samples may be processed simultaneously. In 45 a first variation, the molecular diagnostic module 130 is configured to be stackable with another molecular diagnostic module 130 in a manner that enables access to a microfluidic cartridge 210 within each molecular diagnostic module 130; an example of the first variation is shown in FIG. **6**B, where 50 the molecular diagnostic modules 130 are stacked in a staggered configuration. In the first variation, each molecular diagnostic module 130 may further comprise locking pins or other appropriate mechanisms to couple the stacked molecular diagnostic modules 130 together. In another 55 variation, the molecular diagnostic module 130 may not be configured to stack with another molecular diagnostic module, such that the molecular diagnostic modules 130 are configured to rest side-by-side on the same plane. Elements of an embodiment of the molecular diagnostic module 130 60 are further described in sections 1.2.1 to 1.2.5 below.

# 1.2.1 Molecular Diagnostic Module—Cartridge Receiving Module

As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge platform 141 including a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and

a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141; and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol. As shown in FIGS. 7A-7C, the cartridge platform 141 is preferably configured to receive a microfluidic cartridge 210 along a cartridge loading guiderail 142 until it reaches a cartridge stop 143, and be vertically displaced by the linear actuator 146, which places a biasing force against the set of springs 148 coupled to the cartridge platform 141. The magnet receiving slot 144 and the set of valve actuation slots 145 provide access, by a magnet 160 and a valve actuation subsystem 170, to the microfluidic cartridge 210, as the microfluidic cartridge is vertically displaced by the

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The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment. Alternatively, the cartridge loading guiderails 142 and the cartridge stop 143 may be configured such that a microfluidic cartridge slides over or along the cartridge loading guiderails 142, after which the cartridge stop 143 couples to a portion of the microfluidic cartridge 210 to ensure proper alignment of the microfluidic cartridge. Additional variations of the cartridge loading guiderails 142 and the cartridge stop 143 may be used to enable reception and alignment of a microfluidic cartridge 210 by the molecular diagnostic module 130, and are known by those skilled in

The embodiment of the cartridge platform 141 shown in FIG. 8 also includes a set of valve actuation slots 145, oriented perpendicular to the parallel cartridge loading guiderails 142 and configured to provide access to a valve actuation subsystem 170, and a magnet receiving slot 144 located among the set of valve actuation slots 145. Preferably, the magnet receiving slot 144 and the set of valve actuation slots 145 substantially span a long dimension of the cartridge platform 141, as shown in FIG. 8, and are configured to correspond to locations on a microfluidic cartridge 210 requiring a magnetic field and/or valving to enable processing of a biological sample and nucleic acid detection once the microfluidic cartridge 210 has been aligned within the molecular diagnostic module 130. Thus, alternative configurations of the magnet receiving slot 144 and the set of valve actuation slots 145 may accommodate other cartridges with alternative regions requiring magnetic fields and/or valving to enable other protocols. In one alternative embodiment, the magnet receiving slot 144 and

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the set of valve actuation slots may comprise one continuous void of the cartridge platform 141, such that the cartridge platform 141 supports a microfluidic cartridge 210 along the periphery of the microfluidic cartridge 210, but forms a continuous void under a majority of the footprint of the 5 microfluidic cartridge 210.

The linear actuator 146 functions to linearly displace a microfluidic cartridge 210 resting on the cartridge platform 141, in order to compress the microfluidic cartridge 210 and position the microfluidic cartridge 210 between a cartridge heater 153 and an optical subsystem 180 on one side of the microfluidic cartridge 210, and a magnet 160 and detection chamber heaters 157 on another side of the microfluidic cartridge 210. The linear actuator 146 also functions to provide a sufficient counterforce to the valve actuation 15 subsystem 170 such that a microfluidic cartridge 210 within the molecular diagnostic module 130 remains properly situated upon manipulation by the valve actuation subsystem 170. The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in 20 order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210. In the orientation of the molecular diagnostic module 130 shown in FIGS. 7B and 7B, the linear actuator 146 is preferably coupled to a portion of the heating and cooling subsystem 150 a portion 25 of the optical subsystem 180, and the nozzle 149, and vertically displaces the cartridge heater 153, the optical subsystem 180, and the nozzle 149 to position the cartridge heater 153, 180 and the nozzle 149 over the microfluidic cartridge 210. The vertical displacement also allows the 30 microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the 35 nucleic acid (e.g. PCR). Preferably, the linear actuator **146** is a scissor jack actuator configured to apply substantially uniform pressure over all occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and to operate in at least two configurations. In 40 a retracted configuration 146a, as shown in FIG. 9A, the scissor jack actuator has not linearly displaced the cartridge platform 141, and in an extended configuration 146b, as shown in FIG. 9B, the scissor jack actuator has linearly displaced the microfluidic cartridge 210 to position the 45 microfluidic cartridge 210 between the subsystems 153, and 180, and the magnet 160 and detection chamber heaters 157. Additionally, the extended configuration 146b of the scissor jack actuator is configured to couple the nozzle 149 to a fluid port 222 of the microfluidic cartridge 210, such that the 50 liquid handling system 250 can deliver solutions and gases for processing of biological samples. The linear actuator 146 may alternatively be any appropriate linear actuator, such as a hydraulic, pneumatic, or motor-driven linear actuator, configured to linearly displace a microfluidic cartridge 55 within the molecular diagnostic module 130.

As shown in FIGS. 7B, 7C, and 8, a set of springs 148 is coupled to the cartridge platform 141 and functions to provide a counteracting force against the linear actuator 146 as the linear actuator 146 displaces a microfluidic cartridge 60 210 resting on the cartridge platform 141. The set of springs 148 thus allows the cartridge platform 141 to return to a position that allows the microfluidic cartridge 210 to be loaded and unloaded from the molecular diagnostic module 130 when the linear actuator 146 is in a retracted configuration 146b, as shown in FIG. 7B. Preferably, in the orientation shown in FIG. 7B, the set of springs 148 is located at

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peripheral regions of the bottom side of the cartridge platform 141, such that the set of springs 148 does not interfere with the magnet or the valve actuation subsystem 170. Alternatively, the set of springs 148 may be located at any appropriate position to provide a counteracting force against the linear actuator 146. In a specific example shown in FIG. 6A, the set of springs 148 comprises four springs located near corners of the bottom side of the cartridge platform 141, but in other variations, the set of springs 148 may comprise any appropriate number of springs. Each spring of the set of springs 148 is also preferably housed within a guide to prevent deviations from linear vertical motions (in the orientation shown in FIG. 7B); however, each spring in the set of springs 148 may alternatively not be housed within a guide. In an alternative embodiment of the molecular diagnostic module 130, the set of springs 148 may altogether be replaced by a second linear actuator configured to linearly displace a microfluidic cartridge 210, resting on the cartridge platform 141, in a direction opposite to the displacements enforced by the linear actuator 146, and/or by any other suitable element (e.g., elastomeric element) configured to provide a biasing force against a microfluidic cartridge 210 at the cartridge platform 141.

Similarly, the nozzle 149, the heating and cooling subsystem 150, the cartridge heater 153, and the magnet 160 are preferably coupled to springs, such that springs are positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. Alternatively an elastomeric material is preferably positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. The springs and/or elastomeric material function to provide proper functioning and alignment of subsystems of the molecular diagnostic module 130 as the linear actuator 146 is extended or retracted, contributing to reliability and a reduction in stack up tolerance risk. The springs and/or elastomeric material further function to allow more pressure to be applied to occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and an appropriate pressure to be applied to elements 149, 150, 153 and 160 of the molecular diagnostic module 130. Thus, proper contact is maintained between elements 149, 150, 153, and 160, and a microfluidic cartridge 210 being manipulated by the molecular diagnostic module. These elements are described in further detail below.

 $1.2.2\ Molecular\ Diagnostic\ Module \\ -- Heating/Cooling\ Subsystem\ and\ Magnet$ 

The heating and cooling subsystem 150 of the molecular diagnostic module 130 comprises a cartridge heater 153, a fan 155, and a set of detection chamber heaters 157 and functions to controllably heat portions of a microfluidic cartridge 210 for processing of a biological sample containing nucleic acids according to a molecular diagnostic protocol. In the orientation of an embodiment of the molecular diagnostic module 130 shown in FIGS. 7A-7C, the cartridge heater 153 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140 and configured to span a central region of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, the fan 155 is located at a back wall of the cartridge receiving module 140, and the set of detection chamber heaters 157 is located inferior to a set of detection chambers 213 of the microfluidic cartridge **210**. In alternative embodiments of the molecular diagnostic module 130, the heating and cooling subsystem 150 may have any appropriate alternative configuration that provides controlled heating and cooling to a microfluidic cartridge within the molecular diagnostic module 130.

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The cartridge heater 153 functions to transfer heat to a heating region 224 of a microfluidic cartridge 210, for inducing a pH shift to release bound nucleic acids from magnetic beads within the heating region **224**. The cartridge heater 153 is preferably a plate-shaped heater configured to 5 transfer heat to the microfluidic cartridge 210 only from one side of the cartridge heater 153, such that heat flows through one face of the plate-shaped heater to the microfluidic cartridge 210. In a specific example, the cartridge heater 153 is a silicon wafer etched to be conductive and form a 10 resistance heater. In the preferred variation, the cartridge heater 153 is either flip-chip bonded (i.e., soldered to back side of a circuit board), or wire bonded to a circuit board, and then coupled using linear bearings and springs to a plate coupled to the linear actuator 146. The preferred variation 15 allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing. In another variation, heating through one face is accomplished using a plate-shaped resistance heater that has one exposed face and thermal insulation covering all other faces, 20 and in yet another variation heating through one face is accomplished using a Peltier heater. In a variation of the cartridge heater 153 using a Peltier heater, the cartridge heater 153 comprises a thermoelectric material, and produces different temperatures on opposite faces of the car- 25 tridge heater 153 in response to a voltage difference placed across the thermoelectric material. Thus, when a current flows through the Peltier heater, one face of the Peltier heater lowers in temperature, and another face of the Peltier heater increases in temperature. Alternative variations of the cartridge heater 153 can be used to appropriately transfer heat to a heating region 224 of the microfluidic cartridge 210.

Preferably, the cartridge heater 153 is configured to linearly translate with the linear actuator 146 of the cartridge receiving module 140, in order to align with a heating region 35 224 spanning a central portion of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In one variation, the cartridge heater 153 is preferably fixed relative to the linear actuator 146 such that (in the orientation shown in FIGS. 7B-7C), the cartridge heater 153 can only 40 move vertically with the linear actuator. In an alternative variation, the cartridge heater 153 may additionally be configured to translate laterally with a horizontal plane (in the orientation shown in FIGS. 7B-7C), such that the cartridge heater 153 can translate in at least two perpendicular 45 coordinate planes. In this alternative variation, the cartridge heater 153 can be configured to sweep across a surface of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, or to translate in response to motion of the microfluidic cartridge 210, such that the position of 50 the cartridge heater 153 relative to a heating region 224 of the microfluidic cartridge 210 is always fixed.

The fan 155 functions to modulate heat control within the molecular diagnostic module 130, by enabling heat transfer from warm objects within the molecular diagnostic module 55 130 to cooler air external to the molecular diagnostic module 130. In the orientation shown in FIG. 6A, the fan 155 is preferably located at a back face of the molecular diagnostic module 130, such heat within the molecular diagnostic module 130 is transferred out of the back face of the 60 molecular diagnostic module 130 to cooler air external to the molecular diagnostic module. In a specific embodiment, the molecular diagnostic module 130 comprises four fans 155 located at the back face of the molecular diagnostic module 130; however, in alternative embodiments the molecular 65 diagnostic module 130 may comprise any appropriate number of fans located at any appropriate position of the

molecular diagnostic module 130. In one variation, the fan 155 may be passive and driven solely by convection currents resulting from motion of hot air within the molecular diagnostic module to cooler air outside of the molecular diagnostic module; however, in alternative variations, the fan 155 may be motor driven and configured to actively cool

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diagnostic module; however, in alternative variations, the fan 155 may be motor-driven and configured to actively cool internal components of the molecular diagnostic module 130 if molecular diagnostic module elements exceed a certain

threshold temperature.

The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210. Each detection chamber heater in the set of detection chamber heaters 157 is preferably configured to heat one side of one detection chamber in the set of detection chambers 213, and is preferably located such that the extended configuration **146**b of the linear actuator **146** of the cartridge receiving module 140 puts a detection chamber in proximity to a detection chamber heater. As mentioned above, the set of detection chamber heaters 157 is preferably coupled to springs or an elastomeric layer to ensure direct contact between the set of detection chamber heaters and a set of detection chambers, without compressively damaging the set of detection chamber heater 157. Preferably, each detection chamber heater is configured to contact a surface of a detection chamber in the extended configuration 146b of the linear actuator 146; however, each detection chamber heater may be further configured to couple to a detection chamber in the extended configuration 146b of the linear actuator 146. In a first variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a flexible printed circuit board, with a set of springs coupled to an opposite surface of the flexible printed circuit board, such that each spring in the set of springs aligns with a detection chamber heater. In the first variation, contact between each detection chamber heater and a detection chamber is thus maintained by a biasing force provided by an individual spring through the flexible printed circuit board. In a second variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a rigid printed circuit board, with a set of springs coupled to an opposite surface of the rigid printed circuit board. In the second variation, the set of springs thus function to collectively transfer a force through the rigid printed circuit board to maintain contact between the set of detection chamber heaters and a set of detection chambers. Preferably, each detection chamber heater in the set of detection chamber heaters 157 is configured to contact and heat a bottom surface of a detection chamber (in the orientation shown in FIG. 7B); however, each detection chamber heater may alternatively be configured to contact and heat both a top and a bottom surface of a detection chamber. Additionally, each detection chamber heater preferably corresponds to a specific detection chamber of the set of detection chambers 213 and functions to individually heat the specific detection chamber; however, alternatively, each detection chamber heater may be configured to heat multiple detection chambers in the set of detection chambers 213. Preferably, all detection chamber heaters in the set of detection chamber heaters 157 are identical; however, the set of detection chamber heaters 157 may alternatively not comprise identical detection chamber heaters.

In one variation, each detection chamber heater in the set of detection chamber heaters 157 comprises a donut-shaped heater, configured to encircle a surface of a detection chamber. The donut-shaped heater may further include a conducting mesh configured to allow detection through the heater

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while still allowing efficient heat transfer to the detection chamber. In an alternative variation, each detection chamber heater in the set of detection chamber heaters 157 may include a plate-shaped Peltier heater, similar to Peltier cartridge heater 153 described above. In this alternative 5 variation, each detection chamber heater is thus configured to heat one side of a detection chamber through one face of the detection chamber heater. In one specific example, the molecular diagnostic module 130 comprises 12 diced silicon wafers with conductive channels flip chipped to 12 detection 10 chambers, providing resistive heating to each of the 12 detection chambers. In another specific example, the molecular diagnostic module 130 comprises a 12 Peltier detection chamber heaters configured to heat 12 detection chambers of a microfluidic cartridge 210 aligned within the 15 molecular diagnostic module 130. In other alternative variations, each detection chamber heater may comprise any appropriate heater configured to individually heat a detection chamber.

In some variations, reflection from the set of detection 20 chamber heaters 157 can interfere with light transmitted to photodetectors of the optical subsystem 180 (e.g., light emitted from the set of biological samples, light transmitted through filters of an optical subsystem), especially in configurations wherein the set of detection chambers 213 of a 25 microfluidic cartridge 210 are positioned between detection chamber heaters 157 and optical elements of an optical subsystem 180. In these variations, the set of detection chamber heaters 157 can include elements that reduce or eliminate reflection from the set of detection chamber heaters 157, thereby facilitating analysis of the set of biological samples. In one variation, the set of detection chamber heaters 157 can include or be coupled to non-reflective coatings at surfaces of the set of detection chamber heaters 157 upon which light from the optical subsystem 180 35 impinges. In a specific example, the non-reflective coating can comprise a high-temperature paint (e.g., dark paint, flat paint) that absorbs and/or diffuses light from the optical subsystem 180, while facilitating heat transfer to a set of detection chambers 213 of a microfluidic cartridge 210. In 40 another variation, the set of detection chamber heaters can be in misalignment with photodetectors of the optical subsystem 180, such that reflection does not interfere with light transmitted to the photodetectors of the optical subsystem 180. In one example, the set of detection chamber heaters 45 can be configured to heat a set of detection chambers 213 from a first direction, and the optical subsystem 180 can be configured to receive light from the set of detection chambers 213 from a second direction (e.g., a direction nonparallel to the first direction), such that reflection from the 50 detection chamber heaters 157 does not cause interference. In still other variations, the set of detection chamber heaters 157 can include any other suitable elements (e.g., coatings, layers, etc.) and/or be configured in any other suitable manner that eliminates, prevents, or mitigates reflection 55 of the microfluidic cartridge 210 is located at a surface of the from the set of detection chamber heaters 157 from interfering with light transmitted to photodetectors of the optical subsystem 180.

The magnet 160 of the molecular diagnostic module 130 functions to provide a magnetic field for isolation and 60 extraction of nucleic acids bound to magnetic beads within a microfluidic cartridge 210, aligned within the molecular diagnostic module 130. Preferably, the magnet 160 is fixed within the molecular diagnostic module 130, such that the extended configuration 146b of the linear actuator 146 65 allows the magnet 160 to pass through the magnet receiving slot 144 of the cartridge receiving module 140 and into a

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magnet housing region 218 of the microfluidic cartridge 210. In an example, as shown in FIGS. 7A-7C, the magnet 160 is a rectangular prism-shaped magnet 160 fixed under the cartridge platform 141, and configured to pass through the cartridge platform 141, into a magnet housing region 218 located at a surface of the microfluidic cartridge 210 directly opposing the heating region 224 of the microfluidic cartridge 210. As such, in some variations, a projection of the heating region 224 onto a plane at least partially overlaps with a projection of the magnet housing region 218 onto the plane. However, the magnet housing region 218 and the heating region 224 of the microfluidic cartridge 210 can be in thermal communication in any other suitable manner. Preferably, the magnet 160 is one of multiple magnets (e.g., 2-3 magnets) lined up in parallel, such that each of the fluidic pathways of a microfluidic cartridge housing the magnets is exposed to a multiplied magnetic flux (e.g., two or three times as much magnetic flux), and a multiplied opportunity to capture magnetic beads. Alternatively, the magnet 160 is a single magnet configured to expose a set of fluidic pathways to a magnetic field. Preferably, the magnet 160 or group of multiple magnets is coupled to a magnet holder within the molecular diagnostic module 130. Additionally, the magnet holder is preferably composed of an insulating material, such that the magnet holder does not interfere with proper functioning of the cartridge heater 153. Alternatively, the magnet holder may not be composed of an insulating material.

In one variation, the magnet 160 or group of multiple magnets comprises a permanent magnet, composed of a magnetized material (e.g., a ferromagnet) providing a substantially fixed magnetic field. In an alternative variation, the magnet 160 or group of multiple magnets comprises an electromagnet configured to provide a modifiable magnetic field, such that the intensity of the magnetic field can be adjusted, the polarity of the magnetic field can be reversed, and the magnetic field can be substantially removed upon removal of a current flowing within the electromagnet. Preferably, the magnet 160 or group of magnets is also fixed relative to the molecular diagnostic module 130; however, the magnet 160 or group of magnets may alternatively be configured to translate vertically (in the orientation shown in FIG. 7B), such that the magnet 160 or group of magnets can extend into and retract from the magnet receiving slot 144 of the cartridge platform 141 and the magnet housing region 218 of the microfluidic cartridge 210. Additionally, the magnet 160 or group of magnets preferably rides on linear bearings and/or springs (or an elastomeric material) to ensure proper contact with a microfluidic cartridge in an extended configuration 146b of the linear actuator 146, in a manner that allows most of force from the linear actuator 146 to translate to full occlusion of a subset of the set of occlusion positions (i.e., without leakage).

In some variations, wherein a magnet housing region 218 microfluidic cartridge 210 directly opposing the heating region 224 of the microfluidic cartridge 210, all or a subset of the magnet(s) **160** can be heated, such that the magnet(s) do not provide a heat sink at surfaces of the microfluidic cartridge 210 opposing the cartridge heater 153 and/or any other portion of the microfluidic cartridge 210 intended to have a desired heated state (e.g., a portion of the microfluidic cartridge proximal the set of detection chamber heaters 157). Preferably, heating of the magnet(s) 160 of the molecular diagnostic module is performed in a manner that does disrupt alignment of magnetic domains (e.g., for a permanent magnet), such that a magnetic field provided by the

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magnet(s) 160 does not diminish in strength. As such, a magnet 160 of the molecular diagnostic module 130 is preferably heated to a temperature less than its Curie point, or can additionally or alternatively comprise a magnetic material with a sufficiently high Curie point (e.g., a Curie 5 point characterized by a higher temperature than temperatures required for processing of samples at the microfluidic cartridge 210). In one example, the magnet(s) 160 can thus be configured to be heated to one or more temperatures in synchronization with temperatures of the cartridge heater 10 153, in order to further increase uniformity of heating through the microfluidic cartridge (e.g., from a heating region 224 to a magnet-housing region). The magnet 160 can, however, be any other suitable magnet (e.g., permanent magnet, electromagnet) that is not disrupted by heating 15 within the range of temperatures required for processing of samples at the microfluidic cartridge 210. Furthermore, the magnet(s) 160 of the molecular diagnostic module 130 can be configured to be heated with any suitable temperature output, such that the magnet(s) facilitate generation of any 20 suitable heating profile (e.g., non-uniform heating profile, uniform heating profile, etc.) through the microfluidic cartridge 210.

In one variation, the molecular diagnostic module 130 can comprise at least one magnet 160 coupled to a magnet 25 heating element, such that the magnet heating element heats the magnet 160 to a desired state. In one example of this variation, the molecular diagnostic module 130 can comprise a set of magnets 260, wherein each magnet 160 of the set of magnets 260 is coupled to a magnet heating element 30 **261** at least at one surface of the magnet. As such, the magnet heating element can be coupled to a surface of the magnet 160, can wrap about multiple surfaces of the magnet, can be at least partially embedded in the magnet, and/or can be coupled to the magnet in any other suitable manner. In 35 one specific example, as shown in FIG. 9E, each magnet 160 of the set of magnets 260 is separated from an adjacent magnet by a magnet heating element 261, and in another specific example, each magnet 160 of the set of magnets has a magnet heating element coupled to a distal end of the 40 magnet 160, wherein the distal end of the magnet 160 is configured to interface with the magnet housing region 218 of the microfluidic cartridge 210. The magnet(s) 160 and the magnet heating element(s) 261 of the microfluidic cartridge 210 can, however, be configured in any other suitable 45

Additionally or alternatively, the molecular diagnostic module 130 can be configured with an insulation gap between the magnet(s) 160 and a surface of the microfluidic cartridge 210 proximal the magnet housing region 218, such 50 that the magnet(s) 160 do not interfere with heating of the microfluidic cartridge 210. The insulation gap can be an air gap within the system or can additionally or alternatively comprise any other suitable insulating layer situated between the magnet(s) 160 and the surface of the microfluidic cartridge 210 opposing the cartridge heater 153.

In any of the above embodiments and variations of the magnet(s), the magnet(s) are preferably configured to span a substantial portion of a capture segment 263 (e.g., an s-shaped capture segment with a characteristic width) of the 60 microfluidic cartridge 210, by way of the magnet housing region 218, wherein the capture segment is a portion of a fluidic pathway configured to facilitate capture of target particles bound to magnetic particles. As such, the magnet is preferably substantially wide in order to span a majority of 65 the capture segment and provide a desired gradient of magnetic strength at the capture segment, by way of the

magnet housing region 218 of the microfluidic cartridge 210. Additionally, the strength of the magnet(s) can be adjusted to prevent clogging within the capture segment, for instance, by adjusting morphology, composition, and/or any other suitable characteristic of the magnet(s). In one specific example, the magnet is wide enough to span a majority, but not all, of an s-shaped capture segment 263 of a microfluidic cartridge, by crossing the s-shaped capture segment in an orientation perpendicular to a flow direction through the s-shaped capture segment 163, as shown in FIGS. 9C-9E; however, the magnet(s) 160 can alternatively be configured in any other suitable manner.

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Alternative configurations and/or compositions of the magnet 160 may also be appropriate in facilitating isolation and extraction of nucleic acids bound to magnetic beads within the microfluidic cartridge 210.

1.2.3 Molecular Diagnostic Module—Valve Actuation Subsystem

As shown in FIGS. 10A-11C, the valve actuation subsystem 170 of the molecular diagnostic module 130 comprises a set of pins 172 configured to translate linearly within a pin housing 175, by sliding a cam card 177 laterally over the pins 172. The valve actuation subsystem 170 functions to provide a biasing force to deform an object in contact with the set of pins 172. In a configuration wherein a microfluidic cartridge 210 is aligned within the molecular diagnostic module 130, the valve actuation subsystem 170 thus functions to occlude a fluidic pathway 220 of the microfluidic cartridge 210 at a set of occlusion positions 226, to control flow of a biological sample containing nucleic acids, reagents and/or air through the microfluidic cartridge 210. In an embodiment of the molecular diagnostic module shown in FIGS. 7D-7E, the set of pins 172 and the pin housing are located directly under the microfluidic cartridge 210, such that the set of pins can access the microfluidic cartridge 210 through the valve actuation accommodating slots **145** of the cartridge platform 141. The cam card 177 in the embodiment is positioned under the set of pins and is coupled to a linear cam card actuator 178 configured to laterally displace the cam card 177 to vertically displace pins of the set of pins 172. Preferably, as shown in FIG. 11A, the cam card 177 rests on a low friction surface configured to facilitate lateral displacement of the cam card 177; however, the cam card 177 may alternatively rest on a bed of ball bearings to facilitate lateral displacement of the cam card 177, or may rest on any feature that allows the cam card 177 to be laterally displaced by the linear cam card actuator 178.

The cam card 177, as shown in FIGS. 7D and 11A, includes a set of hills 176 and valleys 179, and functions to transform linear motion in one plane to vertical motion in another plane. In one variation, the cam card 177 is coupled to a linear actuator and contacts the ends of pins in a set of pins 172, such that when a hill 176 of the cam card 177 passes under a pin, the pin is in a raised configuration 177a, and when a valley 179 of the cam card 177 passes under a pin, the pin is in a lowered configuration 177b. The hills 176 and valleys 179 of the cam card 177 are preferably in a set configuration, as shown in FIG. 11B, such that lateral motion of the cam card 177 to a set position raises a fixed subset of the set of pins 172. In this manner, lateral movement of the cam card 177 to different positions of a set of positions consistently raises different subsets of the set of pins 172 to occlude different portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172. Thus, portions of a fluidic pathway 220 may be selectively occluded and opened to facilitate processing of a biological sample according to any appropriate tissue, cellular, or

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molecular diagnostic assay protocol. In one variation, the cam card is configured to be laterally displaced in two coordinate directions within a plane (e.g., by x-y linear actuators), and in another variation, the cam card is configured to be laterally displaced in only one coordinate direction within a plane (e.g., by a single linear actuator). In a specific example, the hills 176 of the cam card 177 are raised 1 mm above the valleys 179 of the cam card 177, the hills 176 and valleys 179 each have a 2 mm wide plateau region, and a hill 176 region slopes down to a valley region 179 at 10 a fixed angle over a 2 mm length. In the specific example, the cam card 177 is driven by a Firgelli linear actuator. Alternative variations may include any appropriate configurations and geometries of a cam card with hills 176 and valleys 179, driven by any appropriate actuator.

In alternative embodiments of the valve actuation subsystem 170, the cam card 177 may be a cam card wheel comprising a set of hills 176 and valleys 179 on a cylindrical surface, and configured to convert rotary motion to linear (i.e., vertical) motion of the set of pins 172. The cam card 20 wheel may be configured to contact ends of pins in the set of pins 172, and may be coupled to a motor shaft and driven by a motor. In other alternative embodiments of the valve actuation subsystem 170, the cam card 177 may altogether be replaced by a set of cams, each configured to individually 25 rotate about an axis. In these alternative embodiments, rotating subsets of the set of cams raises corresponding subsets of the set of pins, and occludes specific portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172.

The set of pins 172 functions to selectively occlude portions of a fluidic pathway 220 of a microfluidic cartridge 210 at least at subsets of a set of occlusion positions 226. The pins of the set of pins 172 are preferably cylindrical and, in the orientation shown in FIG. 11A, configured to slide 35 over a cam card 177 and within a pin housing 175. Each pin in the set of pins 172 preferably also includes a first spring 173 that functions to provide a counteracting force to restore a pin to a lowered configuration 177b; however, each pin in the set of pins 172 may alternative not include a first spring 40 173, and rely solely on gravity to return to a lowered configuration 177b. Preferably, as shown in FIG. 11C, each pin is also composed of two parts separated by a second spring, which functions to allow sufficient force to fully occlude a microfluidic channel but prevents forces from 45 being generated that could damage the pin, microfluidic cartridge and/or cam card. Each pin also preferably comprises a first region 171 configured to slide within the pin housing 175, and a second region 174 configured to exit the pin housing 175. The second region 174 is preferably of a 50 smaller dimension than the first region 171, such that each pin is constrained by the pin housing 175 to be raised by a limited amount. Alternatively, the first region 171 and the second region 174 may have any appropriate configuration to facilitate raising and lowering of a pin by a fixed amount. 55 In a specific example, the valve actuation subsystem 170 comprises 12 sets of pins 172 configured to selectively occlude 12 fluidic pathways 212 of a microfluidic cartridge 210 aligned within the molecular diagnostic module; however, other embodiments may comprise any appropriate 60 number of sets of pins 172.

In the orientation shown in FIG. 11A, each pin in the set of pins 172 preferably has a circular cross section and round ends, configured to facilitate sliding within a pin housing 175, sliding over a cam card 177 surface, and occlusion of 65 a fluidic pathway 220. Alternatively, each pin may comprise any appropriate cross-sectional geometry (e.g., rectangular)

and/or end shape (e.g., flat or pointed) to facilitate occlusion of a fluidic pathway 220. Preferably, the surface of each pin in the set of pins 172 is composed of a low-friction material to facilitate sliding motions (i.e., over a cam card 177 or within a pin housing 175); however, each pin may alternatively be coated with a lubricant configured to facilitate sliding motions.

The pin housing 175 functions to constrain and guide the motion of each pin in the set of pins 172, as the cam card 177 slides under the set of pins 172. Preferably, the pin housing 175 comprises a set of pin housing channels 169 configured to surround at least one pin in the set of pins 172. In one variation, each pin in the set of pins 172 is surrounded by an individual channel of the set of pin housing channels 169; however, in another variation a channel of the set of pin housing channels 169 may be configured to surround multiple pins in the set of pins 172. In an example shown in FIGS. 7D-7E and 11A, the pin housing is located under the cartridge platform 141, such that the set of pin housing channels 169 is aligned with the set of valve actuation accommodating slots 145, to provide access, by the set of pins 172, to a microfluidic cartridge 210 aligned on the cartridge platform 141. In the example, the pin housing 175 thus constrains the set of pins 172, such that each pin can only move linearly in a vertical direction. Each pin housing channel preferably has a constricted region 168 configured to limit the motion of a pin within a pin channel; however, each pin housing channel may alternatively not include a constricted region. Preferably, surfaces of the pin housing 175 contacting the set of pins 172 are composed of a low friction material to facilitate sliding of a pin within a pin housing channel; however, surfaces of the pin housing 175 contacting the set of pins 172 may alternatively be coated with a lubricant configured to facilitate sliding motions. Other variations of the pin housing 175 and the set of pins 172 may include no additional provisions to facilitate sliding of a pin within a pin housing channel.

In some embodiments of the molecular diagnostic module 130, the valve actuation subsystem 170 can be configured in any other suitable manner to facilitate actuation of a set of pins 172 to occlude a microfluidic cartridge 210 at a set of occlusion positions 226. In one embodiment, the valve actuation subsystem 170 can be an embodiment of the valve actuation subsystem described in U.S. application Ser. No. 14/229,396, entitled "System and Method for Processing Biological Samples" and filed on 28 Mar. 2014, which is incorporated herein in its entirety by this reference.

1.2.4 Molecular Diagnostic Module—Optical Subsystem

As shown in FIGS. 12A-12D, the optical subsystem 180 of the molecular diagnostic module 130 comprises a set of light emitting diodes (LEDs) 181, a set of excitation filters 182 configured to transmit light from the set of LEDs 181, a set of dichroic mirrors 183 configured to reflect light from the set of excitation filters 182 toward a set of apertures 185 configured to transmit light toward a set of nucleic acid samples, a set of emission filters 186 configured to receive and transmit light emitted by the set of nucleic acid samples, and a set of photodetectors 187 configured to facilitate analysis of light received through the set of emission filters 186. The optical subsystem 180 may further comprise a set of lenses 184 configured to focus light onto the set of nucleic acid samples. The optical subsystem 180 thus functions to transmit light at excitation wavelengths toward a set of nucleic acid samples and to receive light at emission wavelengths from a set of nucleic acid samples. Preferably, the optical subsystem 180 is coupled to an optical subsystem actuator 188 configured to laterally displace and align the

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optical subsystem 180 relative to a set of nucleic acid samples at a set of detection chambers 213 of the microfluidic cartridge 210, and is further coupled to a linear actuator **146** of the cartridge receiving module **140** to position the optical subsystem 180 closer to the set of nucleic acid 5 samples. Alternatively, the optical subsystem 180 may not be coupled to a linear actuator 146 of the cartridge receiving module 140, and may only be configured to translate laterally in one direction. In a specific example, the optical subsystem 180 comprises a set of 12 apertures, a set of 12 lenses, a set of 12 dichroic mirrors, a set of 12 excitation filters, a set of 12 LEDs, a set of 12 emission filters, and a set of 12 photodetectors. In a variation of the specific example, each set of 12 optical subsystem 180 elements is configured as two sets of 6 apertures, two sets of 6 lenses, 15 two sets of 6 dichroic mirrors, two sets of 6 excitation filters (e.g., 6 excitation filters spanning different wavelengths of light), two sets of 6 LEDs, two sets of 6 emission filters (e.g., 6 emission filters spanning different wavelengths of light, and two sets of 6 photodetectors, such that the optical 20 subsystem 180 includes two sets of identical optical subsystem units, each comprising 6 of each element. The optical subsystem 180 can, however, comprise any suitable number of identical units, in order to increase throughput in the system 100 by decreasing the amount of time it takes to 25 analyze multiple biological samples. For instance, multiple identical units can enable analyses to be performed using a unit of the optical subsystem 180, at a desired point during sample processing (e.g., a point at which a biological sample in a microfluidic cartridge is being heated). In variations of 30 the optical subsystem 180 comprising multiple units, each unit can be configured to move independently of the other units, or can additionally or alternatively be configured to move with the other units in a non-independent manner. For instance, one optical subsystem unit can be configured to 35 move along a first axis (e.g., a horizontal axis) independently of the other optical subsystem unit(s), but can be configured to move along a second axis (e.g., a vertical axis) nonindependently of the other optical subsystem unit(s).

In the specific examples, as shown in FIG. 7A-7E, the 40 optical subsystem 180 is located within the molecular diagnostic module 130 and coupled to the linear actuator 146 of the cartridge receiving module 140, such that, in the extended configuration 146b of the linear actuator 146, the optical subsystem 180 can be positioned closer to a micro- 45 fluidic cartridge 210 aligned within the molecular diagnostic module. Conversely in the specific example, the optical subsystem 180 is positioned away from the microfluidic cartridge 210 in the retracted configuration 146a of the linear actuator 146. In the specific example, the optical 50 subsystem 180 is further coupled to an optical subsystem actuator 188 configured to laterally displace the optical subsystem 180 relative to the microfluidic cartridge 210, such that the optical subsystem 180 can be aligned with a set of detection chambers 213 of the microfluidic cartridge 210. 55

Preferably, the set of LEDs 181 are not all identical but rather chosen to efficiently produce a certain band of wavelengths of light, such that light from the set of LEDs 181 can be filtered to appropriate narrow wavelengths for analysis of nucleic acid samples. Alternatively, all LEDs in the set of 60 LEDs 181 may be identical, and produce white light comprising all wavelengths of visible light that is filtered to produce the desired wavelength, in which case the LEDs may be stationary. Preferably, the set of LEDs 181 includes phosphor-based LEDs, but the set of LEDs 181 may alternatively include any LEDs configured to provide light of the desired range of wavelengths. The LEDs of the set of LEDs

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181 are preferably configured to emit light of wavelengths corresponding to at least one of the set of excitation filters 182, the set of dichroic mirrors 183, and the set of emission filters 186.

The set of excitation filters 182 is configured to align with the set of LEDs 181 in the optical subsystem 180, and functions to transmit light at excitation wavelengths toward the set of dichroic mirrors 183 of the optical subsystem 180. Preferably, the set of excitation filters 182 are not identical excitation filters, but rather chosen to transmit the different desired ranges of excitation wavelengths. Alternatively, all excitation filters of the set of excitation filters 182 are identical, and configured to transmit light having a fixed range of excitation wavelengths. In one variation, the set of excitation filters 182 includes band pass filters, configured to transmit light between two bounding wavelengths, in another variation, the set of excitation filters 182 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of excitation filters 182 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of excitation filters 182 is interchangeable, such that individual excitation filters may be interchanged to provide different excitation wavelengths of light; however, the set of excitation filters 182 may alternatively be fixed, such that the optical subsystem 180 is only configured to transmit a fixed range of excitation wavelengths.

The set of dichroic mirrors 183 is configured to align with the set of excitation filters 182, and functions to receive and reflect light from the set of excitation filters 182 toward the detection chamber, such that light having a range of excitation wavelengths may be focused, through a set of apertures, onto a set of nucleic acid samples. The set of dichroic mirrors 183 also functions to receive and transmit light from a set of emission filters 186 toward a set of photodetectors **187**, which is described in more detail below. All dichroic mirrors in the set of dichroic mirrors 183 are preferably identical in orientation relative to the set of excitation filters 182 and the set of emission filters 186, and configured to reflect and transmit the appropriate wavelengths of light for the given LED. Alternatively, the set of dichroic mirrors 183 may include identical dichroic mirrors, with regard to orientation, light transmission, and light reflection. In a specific example, in the orientation shown in FIG. 12A, the set of excitation filters 182 is oriented perpendicular to the set of emission filters 186, with the set of dichroic mirrors 183 bisecting an angle between two planes formed by the faces of the set of excitation filters 182 and the set of emission filters 186. In the specific example, light from the set of excitation filters is thus substantially reflected at a 90° angle toward the set of apertures 185, and light from the set of emission filters 186 passes in a substantially straight direction through the set of dichroic mirrors 183 toward the set of photodetectors 187. Other variations of the set of dichroic mirrors 183 may include any configuration of dichroic mirrors, excitation fillers, and/or emission filters that enable transmission of light of excitation wavelengths toward a set of nucleic acid samples, and transmission of light from the set of nucleic acid samples toward a set of photodetectors 187

In one embodiment, the optical subsystem may further include a set of lenses **184** configured to align with the set of dichroic mirrors **183**, which functions to focus light, from the set of excitation filters **182** and reflected off of the set of dichroic mirrors **183**, onto a set of nucleic acid samples configured to emit light in response to the light from the set of excitation filters **182**. All lenses in the set of lenses **184** 

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are preferably identical in orientation relative to the set of dichroic mirrors and in dimension; however, the set of lenses 184 may alternatively comprise non-identical lenses, such that light passing through different lenses of the set of lenses **184** is focused differently on different nucleic acid samples. In a specific example, in the orientation shown in FIG. 12A. the faces of the set of lenses 184 are oriented perpendicular to the faces of the set of excitation filters 182, to account for light reflection by the set of dichroic mirrors 183 at a 90° angle. In the specific example, the set of lenses also includes identical 1/4" high numerical aperture lenses. In other variations, the set of lenses 184 may be oriented in any appropriate configuration for focusing light from the set of dichroic mirrors 183 onto a set of nucleic acid samples, and 15 may include lenses of any appropriate specification (i.e., numerical aperture).

The set of apertures 185 is located on an aperture substrate 189 and configured to align with the set of lenses 184, and functions to allow focused light from the set of lenses **184** 20 to pass through to the set of nucleic acid samples. The aperture substrate 189 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140, which allows the optical subsystem 180 to linearly translate and be positioned near and away from a microfluidic cartridge 210 25 aligned within the molecular diagnostic module 130. Alternatively, the aperture substrate 189 may not be coupled to the linear actuator 146 of the cartridge receiving module 140. Preferably, all apertures 185 in the set of apertures 185 are identical, and configured to allow identical light profiles 30 to be focused, through the set of lenses 184, onto a set of nucleic acid samples. Alternatively, the set of apertures 185 may not include identical apertures. In one variation, each aperture in the set of apertures 185 may be individually adjustable, in order to provide individually modifiable aper- 35 ture dimensions (e.g., width, length, or diameter) to affect light exposure. In an alternative variation, each aperture in the set of apertures 185 is fixed. Other variations may include interchangeable aperture substrates 189, such that features of the set of apertures (e.g., aperture dimensions, 40 number of apertures) may be adjusted by interchanging aperture substrates 189.

The set of emission filters 186 is configured to align with the set of dichroic mirrors, and functions to transmit emission wavelengths of light from the set of nucleic acid 45 samples, and to filter out excitation wavelengths of light. Preferably, each emission filter of the set of emission filters 186 are configured to transmit light having a fixed range of emission wavelengths, while blocking light of excitation wavelengths. Alternatively, the set of emission filters 186 50 may comprise identical emission filters, such that individual emission filters of the set of emission filters 186 are configured to transmit the same ranges of emission wavelengths. In one variation, the set of emission filters 186 between two bounding wavelengths, in another variation, the set of emission filters 186 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of emission filters 186 includes long pass filters configured to transmit light above 60 a certain wavelength. Preferably, the set of emission filters **186** is interchangeable, such that individual emission filters may be interchanged to transmit and/or block different wavelengths of light; however, the set of emission filters 186 may alternatively be fixed, such that the optical subsystem 65 **180** is only configured to transmit a fixed range of emission wavelengths.

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The set of photodetectors 187 is configured to align with the set of emission filters 186, and functions to receive light from the set emission filters to facilitate analysis of the set of nucleic acid samples. All photodetectors in the set of photodetectors 187 are preferably identical; however, the set of photodetectors 187 may alternatively include non-identical photodetectors. Preferably, the set of photodetectors 187 includes photodiodes comprising a photoelectric material configured to convert electromagnetic energy into electrical signals; however, the set of photodetectors 187 may alternatively comprise any appropriate photodetectors for facilitating analysis of biological samples, as is known by those skilled in the art.

The optical subsystem actuator 188 is coupled to the optical subsystem 180, and functions to laterally translate the optical subsystem 180 relative to a set of nucleic acid samples being analyzed. Preferably, the optical subsystem actuator 188 is a linear actuator configured to translate the optical subsystem 180 in one dimension; however, the optical subsystem actuator 188 may alternatively be an actuator configured to translate the optical subsystem 180 in more than one dimension. In a specific example, as shown in FIGS. 7A-7D and 12D, the optical subsystem actuator 188 is configured to translate the optical subsystem 180 laterally in a horizontal plane, to align the optical subsystem 180 with a set of detection chambers 213 of a microfluidic cartridge 210 within the molecular diagnostic module 130. In another example, the optical subsystem may be configured as a disc revolving around an axis with the LEDs and photodetectors stationary and the disc containing the filters. In other variations, the optical subsystem actuator 188 may be configured in any appropriate manner to facilitate alignment of the optical subsystem 180 relative to a set of nucleic acid samples being analyzed.

In some variations, wherein reflection from the set of detection chamber heaters 157 and/or any other element of the system interferes with light emitted directly from biological samples at the microfluidic cartridge 210 or light transmitted through the set of excitation filters 182, the optical system can be configured to filter out undesired reflected light, by way of any one or more of: the set of excitation filters 182, the set of dichroic mirrors 183, the set of emission filters 186, and any other suitable element configured to reduce or remove interference caused by undesired reflected light.

The optical subsystem 180 can, however, comprise any other suitable element(s) and/or be configured in any other suitable manner to facilitate analysis of a set of biological samples.

1.2.5 Molecular Diagnostic Module—Alternative Embodiments and Variations

As described above, alternative embodiments of the includes band pass fillers, configured to transmit light 55 molecular diagnostic module 130 and alternative variations of subsystems and elements of the molecular diagnostic module 130 may be configured to process a biological sample containing nucleic acids, isolate nucleic acids from the biological sample, and detect nucleic acids. An example of an alternative embodiment of a molecular diagnostic module 130, as shown in FIG. 13, includes a cartridge receiving module 140', a heating and cooling subsystem 150', a magnet 160', a valve actuation subsystem 170', and an optical subsystem 180', and functions to manipulate an alternative microfluidic cartridge 210' for processing of biological samples containing nucleic acids. Other alternative embodiments of the molecular diagnostic module 130"

may be configured to receive alternative microfluidic cartridges 210", for processing of biological samples containing

1.3 System—Assay Strip

nucleic acids.

As shown in FIGS. 18A and 18B, the assay strip 190 5 comprises an assay strip substrate 191 comprising a set of wells 192, and typically a puncturable foil seal 195, and functions to facilitate combination of a set of nucleic acid samples with a set of molecular diagnostic reagents for amplification and/or detection of a nucleic acid sequence or 10 sequences. Preferably, the entire assay strip 190 is configured to be a consumable (i.e., disposable), such that the assay strip 190 can be used during multiple runs of the system 100, then the assay strip 190 is disposed of once all of the wells 192, containing unitized reagents for a single 15 test or group of tests, is exhausted. Alternatively, at least a portion of the assay strip 190 is configured to be reusable, such that wells may be reloaded with reagents and reused with the system 100. In one variation of the assay strip 190, the assay strip substrate **191** is reusable, while the punctur- 20 able foil seal 195 is disposable and replaced after each run of the system 100. In another variation, the reusable assay strip substrate 191 does not require a puncturable foil seal 195, such that reagents specific to a certain nucleic acid sequences may be deposited into open wells of the assay 25 strip substrate 191 by a user.

The assay strip substrate 191 is configured such that the assay strip 190 is capable of resting on a flat surface, and functions to define the set of wells 192 and to couple to the puncturable foil seal 195. The assay strip substrate 191 is 30 preferably configured to be received by a corresponding assay strip holder 230 configured to hold multiple assay strips 190, but may alternatively not be configured to couple to an assay strip holder 230. The assay strip substrate 191 is preferably composed of a PCR-compatible polymer, such as 35 polypropylene, that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 192 of the assay strip substrate 191 40 function to receive at least one nucleic acid sample, and to facilitate combination of the nucleic acid sample with at least one of a set of molecular diagnostic reagents. The molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprise reagents configured to 45 analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group 50 B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to perform alternative molecular diagnostic protocols. Preferably, the wells 193 of the assay strip substrate 191 are each configured to accommodate not only a nucleic acid sample, 55 but also to facilitate mixing of the nucleic acid sample with at least one of a set of molecular diagnostic reagents (e.g., using a pipettor or other apparatus). Additionally, the molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprises probes and primers to 60 detect the sample process controls provided by the capture plate, in order to verify process fidelity and assay accuracy. Preferably, the wells 193 are deep enough to facilitate mixing without splashing, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological 65 samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wide and shallow to facilitate drying of reagents in

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the wells to increase shelf life and larger devices for mixing the nucleic acids with molecular diagnostic reagents. Each well 193 of the set of wells 192 also preferably has a rounded bottom region, as shown in FIG. 18A, to facilitate complete aspiration of a fluid from a well 193; however, each well 193 may alternatively not have a rounded bottom region. Additionally, the set of wells 192 is preferably arranged in staggered rows, which functions to facilitate access to individual wells 193 of the set of wells, to reduce one dimension of the assay strip 190, and also to prevent cross-contamination of fluids within the wells due to dripping. Alternatively, the set of wells 192 may not be arranged in staggered rows.

The puncturable foil seal 195 functions to protect the molecular diagnostic reagents stored in wells 112 from degradation, isolate each well 193 of the set of wells 192, prevent contamination of the contents of each of the set of wells 192, and provide information identifying the assay strip 190. The puncturable foil seal 195 preferably seals each well 193 of the assay strip 190, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one variation, the puncturable foil seal 195 also forms a seal around an element that punctures it, and in another variation, the puncturable foil seal 195 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 195 is also preferably labeled with identifying information including at least one of manufacturer information, assay strip contents, the lot of the contents, an expiry date, and a unique electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal 195 does not extend beyond the footprint of the assay strip 190, but alternatively, the puncturable foil seal 195 may be any appropriate size and/or include protruding features (e.g., tabs) that facilitate handling of the assay strip.

In one variation, the assay strip 190 may be prepackaged with a set of molecular diagnostic reagents, such that each well 193 in the set of wells 192 is prepackaged with a quantity of molecular diagnostic reagents. The set of wells 192 may then be sealed by the puncturable foil seal 195, which is configured to be punctured by an external element that delivers volumes of nucleic acid samples to be combined with the set of molecular diagnostic reagents. In another variation, the assay strip 190 may not be prepackaged with a set of molecular diagnostic reagents, and the wells 193 of the assay strip 190 may not be sealed with a puncturable foil seal 195. In yet another variation, the system may comprise an empty assay strip 190 without a puncturable foil seal 195, and an assay strip 190 comprising reagents and a puncturable foil seal 195, such that a user may add specific reagents to the empty assay strip to be used in conjunction with the assay strip comprising reagents. In variations comprising a puncturable foil seal 195, the puncturable foil seal 115 is configured to be punctured by at least one external element, for co-delivery of nucleic acid samples and molecular diagnostic reagents intended to be combined.

In a specific example, the assay strip 190 has an 87 mm×16 mm footprint and comprises 24 wells 113 arranged in two staggered rows, with a 9 mm center-to-center pitch between adjacent wells 193 within each row. Each well 193 of the set of wells has a capacity of 60 μL to accommodate a volume of a molecular diagnostic reagent, 20 μL of a sample fluid, and any displacement caused by a pipette tip (e.g., 100 or 300 μL pipette tip). Each well 113 of the assay strip 190 in the specific example is also prepackaged with a quantity of molecular diagnostic reagents, and comprises a protruding top edge (75 microns high) that is heat sealed to

a puncturable foil seal. The capture plate no in the specific example is produced by injection molding, has a footprint of

127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier. In the specific embodiment, the vapor barrier is further 5 increased by depositing a thin metallic layer to the outside

of the assay strip 190.

As described earlier, the assay strip 190 may be configured to be received by an assay strip holder 230. The assay strip holder 230 functions to receive and align multiple assay strips 190, such that a multichannel pipettor or other fluid delivery system may combine multiple nucleic acid samples with molecular diagnostic reagents using wells 193 of multiple assay strips 190. In one variation, the assay strip holder 230 may be configured to contain Assay strips 190 15 including reagents for substantially different molecular diagnostic assays, as shown in FIG. 17B, such that a single run of the system 100 involves analyzing a set of nucleic acid samples under different molecular diagnostic assays. In another variation, the assay strip holder 230 may be con- 20 figured to contain assay strips 190 including reagents for identical molecular diagnostic assays, such that a single run of the system 100 involves analyzing a set of nucleic acid samples under the same molecular diagnostic assay. Preferably, the assay strip holder 230 is composed of a material 25 that is dishwasher safe and autoclavable, configured to hold the assay strips 190 in place during handling by a fluid delivery system (e.g., pipettor), and configured such that the assay strips 190 avoid protruding over an edge of the assay strip holder 230, but the assay strip holder 230 is constructed 30 to facilitate insertion and removal of the assay strips 190 from the assay strip holder 230.

In one variation, the assay strip holder 230 is not configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190; however, in another variation as 35 shown in FIG. 21A, the assay strip holder 230 may be further configured to couple to an aluminum block 235 coupled to a set of Peltier units 236 configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190. Additionally, the assay strip holder 230 may be 40 configured to be received and carried by an assay strip carrier 240, which, as shown in FIG. 20, functions to facilitate handling and alignment of multiple assay strip holders 230. In a specific example, as shown in FIG. 19, the assay strip holder 230 has dimensions of 127.76 mm×85.48 45 mm×14.35 mm, complies with American National Standards Institute (ANSI) and Society for Laboratory Automation and Screening (SLAS) standards, and is configured to hold six 16-well assay strips for a total of 96 wells 193. In another specific example, as shown in FIG. 21B, the assay 50 strip holder 230' is configured to hold four assay strips 190', each comprising 24 wells 193' for a total of 96 wells per assay strip holder 230'. Other combinations of the described embodiments, variations, and examples of the assay strip 190, assay strip holder 230, and assay strip carrier 240 may 55 be incorporated into embodiments of the system 100 for processing and detecting nucleic acids.

#### 1.4 System—Microfluidic Cartridge

The microfluidic cartridge 210 functions to receive a set of magnetic bead-samples, facilitate separation of nucleic 60 acids from the set of magnetic bead-samples, receive a set of nucleic acid-reagent samples, and facilitate analysis of nucleic acids from the set of nucleic acid-reagent samples. In one embodiment, the microfluidic cartridge 210 comprises a top layer 211 including a set of sample port-reagent 65 port pairs 212 and a set of detection chambers 213; an intermediate substrate 214, coupled to the top layer 211 and

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partially separated from the top layer 211 by a film layer 215, configured to form a waste chamber 216; an elastomeric layer 217 partially situated on the intermediate substrate 214; a magnet housing region 218 accessible by a magnet 160 providing a magnetic field; and a set of fluidic pathways 219, each formed by at least a portion of the top layer 211, a portion of the film layer 215, and a portion of the elastomeric layer 217. In the embodiment, the microfluidic cartridge 10 further comprises a bottom layer 221 coupled to the intermediate substrate 214 and configured to seal the waste chamber 216. Furthermore, in the embodiment, the top layer 211 of the microfluidic cartridge 210 further comprises a shared fluid port 222, a vent region 223, and a heating region 224, such that each fluidic pathway 220 in the set of fluidic pathways 219 is fluidically coupled to a sample port-reagent port pair 224, the shared fluid port 222, the waste chamber 216, and a detection chamber 225, comprises a turnabout portion 226 configured to pass through the heating region 224 and the magnetic field, and is configured to pass through the vent region 223 upstream of the detection chamber 225. Each fluidic pathway 220 thus functions to receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 220.

The microfluidic cartridge 210 is preferably configured to be received and manipulated by the molecular diagnostic module 130, such that the cartridge receiving module 140 of the molecular diagnostic module 130 receives and aligns the microfluidic cartridge 210 within the molecular diagnostic module 130, the heating and cooling subsystem 150 of the molecular diagnostic module 130 is configured to transfer heat to the heating region 224 of the microfluidic cartridge 210, and the magnet 160 of the molecular diagnostic module 130 is configured to be received by the magnet housing region 218 of the microfluidic cartridge 210 to provide a magnetic field for separation of nucleic acids. Additionally, the shared fluid port 222 of the microfluidic cartridge 210 is configured to couple to a nozzle 149 coupled to the linear actuator 146 of the cartridge receiving module 140, such that the liquid handling system 250 can deliver fluids and gases through the shared fluid port 222. The elastomeric layer 217 of the microfluidic cartridge 210 is also preferably configured to be occluded at a set of occlusion positions 226 by the valve actuation subsystem 170 of the molecular diagnostic module, in order to occlude portions of a fluidic pathway 220 of the microfluidic cartridge 210 for processing of a set of biological samples. The optical subsystem 180 of the molecular diagnostic module 130 is further configured to align with the set of detection chambers 213 of the microfluidic cartridge 210, to facilitate analysis of a set of nucleic acid samples. The microfluidic cartridge 210 is preferably the microfluidic cartridge 210 described in U.S. application Ser. No. 13/765,996, which is incorporated in its entirety by this reference, but may alternatively be any appropriate cartridge or substrate configured to receive and process a set of samples containing nucleic acids.

#### 1.5 System—Fluid Handling System and Filter

The liquid handling system 250 of the system 100 includes a liquid handling arm 255 and a syringe pump 265, as shown in FIGS. 14A-14C and functions to deliver biological samples, reagents, and gases to elements of the system 100. As described in Section 1, an embodiment of the liquid handling system 250 is configured to aspirate a set of biological samples containing nucleic acids (i.e., impure nucleic acid samples), dispense the set of biological samples into a capture plate 110 to be lysed and combined with magnetic beads by a capture plate module 120, aspirate the

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set of biological samples combined with magnetic beads (i.e., set of magnetic bead-samples) from the capture plate no, and dispense the set of magnetic bead-samples into microfluidic cartridge 210 located in a molecular diagnostic module 130. The embodiment of the liquid handling system 5 100 is further configured to facilitate separation of a set of nucleic acids from the magnetic bead-samples, by dispensing a wash solution, a release solution, and/or air into the molecular diagnostic module 130, by the nozzle 149 coupled to the linear actuator 146, at appropriate stages, aspirate the 10 set of nucleic acids from the molecular diagnostic module 130, combine the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, and dispense the set of nucleic acids combined with the set of molecular diagnostic reagents (i.e., set of nucleic acid-reagent mix- 15 tures) into the molecular diagnostic module 130 for further processing and analysis. Other embodiments of the liquid handling system 250 may be configured to perform alternative molecular diagnostic assay protocols and/or dispense and aspirate alternative fluids into and from other elements 20 supporting a molecular diagnostic protocol.

The liquid handling arm 255 comprises a gantry 256 and a multichannel liquid handling head 257, and functions to travel to different elements of the system 100 for fluid delivery and aspiration. The liquid handling arm 255 is 25 preferably automated and configured to move, aspirate, and deliver fluids automatically, but may alternatively be a semi-automated liquid handling arm 255 configured to perform at least one of moving, aspirating, and delivering automatically, while another entity, such as a user, performs 30 the other functions.

The gantry 256 is coupled to the multichannel liquid handling head 257, and functions to transport the multichannel liquid handling head 257 to different elements of the system 100 for fluid delivery and aspiration. Preferably, the 35 gantry 256 is automated and configured to translate the multichannel liquid handling head 257 within at least two dimensions, and provides X-Y positional accuracy of at least 0.5 mm. Additionally, in the orientation shown in FIG. 14B, the gantry is preferably situated above the molecular diagnostic module 130, such that the gantry 256 can translate within at least two dimensions without interfering with other elements of the system 100. Alternatively, the gantry 256 may be any appropriate gantry 256 to facilitate movement of an end effector within at least two dimensions, as is readily 45 known by those skilled in the art.

The multichannel liquid handling head 257 functions to aspirate fluids from and deliver fluids to different elements of the system 100. Preferably, the multichannel liquid handling head 257 is a multichannel pipette head; however, the 50 multichannel liquid handling head 257 may alternatively be any appropriate multichannel liquid handling head configured to deliver fluids and/or gases. Preferably, the multichannel liquid handling head 257 comprises at least eight independent channels 258, but may alternatively comprise 55 any number of channels 258 configured to aspirate and deliver fluids. The channel-to-channel pitch is preferably variable, and in a specific example ranges between 9 mm and 36 mm; however, the channel-to-channel pitch may alternatively be fixed, as shown in FIG. 15. The multichan- 60 nel liquid handling head 257 also preferably provides independent z-axis control (in the orientation shown in FIG. 14B), such that, in combination with the gantry 256. The multichannel liquid handling head 257 is preferably configured to couple to both large (e.g., 1 mL) and small (e.g., 65 between 100 and 300 µL) pipette tips, and in a specific example, has a precision of at least 6% using small dispos-

able pipette tips and a precision of at least 2% using large disposable pipette tips when dispensing essentially the entire tip volume. Alternatively, the multichannel liquid handling head 257 may be configured to couple to any object configured to facilitate aspiration and delivery of fluids. Preferably, the multichannel liquid handling head 257 provides independent control of the channels 258, with regard to volumes of fluid aspirated or delivered, fluid dispensing rates, and/or engaging and disengaging pipette tips. Alternatively, the multichannel liquid handling head 257 may not provide independent control of the channels 258, such that all channels 258 of the multichannel liquid handling head 257 are configured to perform identical functions simultaneously. Preferably, the multichannel liquid handling head 257 is configured to aspirate and deliver both liquids and gases, but alternatively, the multichannel liquid handling head 257 may be configured to only aspirate and deliver liquids. Preferably, the multichannel liquid handling head 257 provides at least one of liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258; however, the multichannel liquid handling head 257 may alternatively not provide liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258.

In one embodiment, the multichannel liquid handling head 257 is configured to couple to at least one filter 260, which functions to pre-filter liquids being aspirated and/or dispensed by the liquid handling arm 255, and is preferably a custom filter 260 configured to couple to a pipette tip, but may alternatively be any appropriate filter configured to couple to the liquid handling arm 255 and filter liquids being aspirated and/or dispensed by the liquid handling arm 255.

An embodiment of a custom filter 260, as shown in FIG. 22, comprises a first end 261 configured to couple to a pipette tip, a pointed second end 262, a void 263 coupled to the first end 261 and the pointed second end 262, and a filter membrane 264 subdividing the void 263. The first end 261, as shown in FIG. 22, preferably comprises a tapered channel configured to provide a friction fit with a pipette tip; however, the first end may alternatively not comprise a tapered channel and may be configured to couple to a pipette tip using any appropriate means. The pointed second end 262 is preferably sharp and configured to pierce an object, such as a foil seal; additionally, the pointed second end 262 is preferably at least as long as required to dispense into a well 113 of the capture plate 110. The void 263 preferably defines a conical region defined by the filter membrane 264, wherein the conical region is configured to divert a fluid within the filter 260 toward the pointed second end 262; however, the void 263 may not include a conical region. The filter membrane 264 functions to filter a fluid aspirated by the multichannel liquid handling head 257, and is configured to subdivide the void 263 to define a conical region; however, the filter membrane 264 may alternatively not define a conical region of the void 263. In one embodiment, in the orientation shown in FIG. 22, the region of the void 263 below the filter membrane 264 may have a volumetric capacity of between 200 ul and 1 mL; however, the region of the void 263 below the filter membrane may alternatively have any appropriate volumetric capacity.

A set of filters 260 may further be configured to be received and delivered by a filter holder 269, as, shown in FIG. 23. A specific embodiment of a filter holder 269 comprises a set of 24 tapered holes with an 18 mm center-to-center pitch, arranged in six rows of four holes. The specific embodiment of the filter holder 269 is also compliant with ANSI and SLAS standards, has dimensions of

127.75×85.5×14.35 mm, and is stackable with other specific embodiments of the custom filter holder **269**. Alternatively, the filter holder **269** may be any appropriate filter holder **269** configured to receive and deliver a set of filters **260**, as is

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1.5.1 Fluid Handling System—Syringe Pump

readily known by those skilled in the art.

The syringe pump 265 of the liquid handling system 250 is coupled to a wash solution source 266, a release solution source 267, a source of air 268, and flexible tubing 291, and functions to deliver a wash solution, a release solution, and 10 air through a valve to the molecular diagnostic module 130 to facilitate isolation and purification of nucleic acids from a set of magnetic bead-samples. The flexible tubing 291 is preferably coupled at a first end to the syringe pump, and at a second end to a nozzle 149 coupled to the linear actuator 15 146 of the molecular diagnostic module 130, as shown in FIG. 14C. As stated earlier, an extended configuration 146b of the linear actuator 146 is configured to couple the nozzle 149 to a fluid port 222 of a microfluidic cartridge 210 within the molecular diagnostic module 130, such that the wash 20 solution, release solution, and air can be delivered to the microfluidic cartridge 210 at appropriate stages. A specific embodiment of the syringe pump 265 comprises a 4-way valve, is able to pump 20-5000 µL of fluids or air through the 4-way valve at flow rates from 50-500 μL/min, can couple 25 to syringes with between 1 mL and 10 mL capacities, and has a precision of at least 5% with regard to fluid or air delivery. Alternatively, the syringe pump 265 may be any appropriate syringe pump 265 or fluid delivery apparatus configured to deliver a wash solution, a release solution, and 30 air to the molecular diagnostic module 130, as is readily known by those skilled in the art.

## 1.6 System—Additional Elements

The system 100 may further comprise a tag reader 271, which functions to read barcodes, QR codes and/or any other 35 identifying tags of the system 100. Preferably, the tag reader 271 is coupled to the liquid handling system 250, such that the tag reader 271 is configured to read tags on puncturable foil seals 115, 195 or tags located on any element of the system 100 accessible by the liquid handling system 250; 40 however, the tag reader 271 may alternatively not be coupled to the liquid handling system 250. In one alternative embodiment of the system 100, the tag reader 271 may be a standalone unit that is configured to be manipulated by a user to scan tags or labels located on elements of the system 45 100.

The system 100 may also further comprise a controller 272 coupled to at least one of the capture plate module 120, the molecular diagnostic module 130, the liquid handling system 250, and the tag reader 271, and functions to facili- 50 tate automation of the system 100. In a variation wherein the controller 272 is coupled to the capture plate module 120, the controller 272 preferably functions to automate heating of a capture plate 110, which facilitates lysing of biological samples within the capture plate no and binding of nucleic 55 acids within the capture plate no to magnetic beads 119 of the capture plate 110. In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples 60 within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of nucleic acid-reagent mixtures by the optical subsystem 180. In a variation wherein the controller 272 is coupled to the 65 liquid handling system 250, the controller 272 preferably functions to automate aspiration, transfer, and delivery of

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fluids and/or gases to different elements of the system 100. In a variation wherein the controller 272 is coupled to the tag reader 271, the controller preferably functions to automate reading of tags by the tag reader 271, and may further function to facilitate transfer of information from the tags to a processor 273. Other variations of a controller may function automate handling, transfer, and/or storage of other elements of the system 100, such as capture plates 110, assay strips 190, assay strip holders 230, assay strip carriers 240, filters 200, filter holders 205, and/or microfluidic cartridges 210, using a robotic arm or gantry similar to that used in the liquid handling system 250. Alternative combinations of the above variations may involve a single controller 272, or multiple controllers configured to perform all or a subset of the functions described above.

The system 100 may also further comprise a processor 273, which functions to receive and process information from a tag reader 271, and also to receive and process data received from the optical subsystem 180 of the molecular diagnostic module 130. Preferably, the processor 273 is coupled to a user interface 274, which functions to display processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information. Alternatively, the processor 273 is not coupled to a user interface 274, but comprises a connection 275 configured to facilitate transfer of processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information to a device external to the system 100.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made the described embodiments of the system 100 without departing from the scope of the system 100.

2. Method for Processing and Detecting Nucleic Acids

An embodiment of a method 400 for processing and detecting nucleic acids from a set of biological samples comprises: combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of magnetic bead-sample mixtures S410; heating the set of magnetic bead-sample mixtures to produce a set of nucleic acid-magnetic bead samples S420; transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways S430; producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples S440; combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures S450; transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers S460; and receiving light from the set of nucleic acid-reagent mixtures S470. The method 400 may further comprise generating a set of data based on light received form the set of nucleic acid-reagent mixtures S480. The method 400 functions to isolate and extract a set of nucleic acid volumes from a biological sample, and to facilitate analysis of the nucleic acid volumes according to at least one molecular diagnostic protocol.

Step S410 recites combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of magnetic bead-sample mixtures, and functions to prepare a set of biological samples to be

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lysed and combined with magnetic beads. For each biological sample, Step S410 preferably comprises aspirating a portion of the volume of the biological sample from a sample container (possibly containing an aqueous solution prior to addition of biological sample), and transferring the 5 portion of the biological sample to a well containing a set of magnetic beads. Alternatively, for each biological sample, Step S410 may comprise aspirating the entire volume of the biological sample from a sample container, and transferring the volume of the biological sample to be combined with a 10 set of magnetic beads. Preferably, all biological samples in the set of biological samples are aspirated and combined with the magnetic beads in the wells simultaneously using a multichannel fluid delivery system; however, all biological samples in the set of biological samples may alternatively be 15 aspirated and combined with a set of magnetic beads nonsimultaneously. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively 20 charged. However, the magnetic beads may alternatively be any appropriate magnetic beads configured to facilitate biomagnetic separation.

In addition to combination with magnetic beads, Step **410** may further include combining each biological sample of the 25 set of biological samples with a lysing enzyme (e.g. proteinase K), and a sample process control comprising two or more nucleic acid sequences (i.e., one for DNA and one for RNA) to be included with each sample. This allows biological samples to effectively lysed, which releases waste 30 components into a wash solution, and allows nucleic acids to bind to magnetic beads. This additionally allows the sample process control to be later detected, as a check to verify the accuracy of a molecular diagnostic assay being performed.

In a first variation of Step S410 for one biological sample, as shown in FIG. 16A, a volume of the biological sample is aspirated and combined with a set of magnetic beads. In the first variation of Step S410, a set of different biological samples may thus be aspirated simultaneously, and each 40 biological sample may be transferred to an individual well to be combined with a set of magnetic beads to produce a set of magnetic bead-sample mixtures. In the first variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic bead-sample mixtures are substantially non-iden- 45 tical in composition. In a second variation of Step S410, as shown in FIG. 16B, a volume of a stock biological sample is aspirated, and portions of the volume of the stock biological sample are transferred to multiple wells to be combined with multiple sets of magnetic beads to produce a set 50 of magnetic bead-sample mixtures. In the second variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic bead-sample mixtures are substantially identical in composition. Other variations of Step S410 may additionally or alternatively comprise filtering at least one bio- 55 logical sample of the set of biological samples S415 prior to combining each biological sample of the set of biological samples with a quantity of magnetic beads.

In a specific example of Step S410, a multichannel liquid handling system aspirates approximately 1 mL of each of a 60 set of biological samples in aqueous buffer using a set of 1 mL pipette tips, couples each of the pipette tips to a custom 13 mm diameter filter, punctures a foil seal 115 of a capture plate at a set of wells, wherein each well of the set of wells contains a set of magnetic beads, and dispenses each aspirated volume of a biological sample into a well of the capture plate containing a set of magnetic beads, and dis-

poses of the tip/filter combination. In the specific example of Step S410, the multichannel liquid handling system then picks up new disposable tips and aspirates and dispenses the contents of each well of the set of wells of the capture plate at least three times to mix the contents, and then disposes of the set of pipette tips and filters.

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Additionally or alternatively, the method 400 can include combining each biological sample of the set of biological samples with buffer solution S405 prior to combining each biological sample of the set of biological samples with a quantity of magnetic beads, which functions to further decrease sample preparation burden on an entity processing and/or analyzing the set of biological samples. Combining each biological sample of the set of biological samples with buffer solution can be performed using a buffer plate comprising a set of wells, each well containing buffer solution, wherein the buffer plate is included in an embodiment of the system 100 described above; however, Block S405 can additionally or alternatively be implemented using any other suitable portion of the system 100 described above. In one variation, a liquid handling system can be automatically configured to aspirate the set of biological samples and mix the biological samples with the buffer solution within the buffer plate, by dispensing the biological samples into the buffer plate and aspirating and delivering the samples combined with buffer one or more times. In another variation, combination of the set of biological samples with buffer solution can be implemented by the liquid handling system away from the buffer plate, for instance, by aspirating the set of biological samples, aspirating the buffer solution, and then mixing the biological samples with the buffer solution within the liquid handling system. In other variations, the biological samples can be combined with buffer solution in any other suitable manner (e.g., altogether with mixing the biological sample with magnetic beads). In relation to the specific example of Block S410 described above, the multichannel liquid handling system can be configured to aspirate each of the set of biological samples with a set of pipette tips, dispense each of the set of biological samples into a corresponding well of a set of wells containing buffer solution, mix each of the set of biological samples with the buffer solution using the set of pipette tips, while preventing cross contamination across the set of samples by way of the set of pipette tips, couple the set of pipette tips to a custom filter, and push the biological samples mixed with buffer solution into wells of a capture plate, each well of the capture plate containing a set of magnetic beads. As such, mixing of biological samples with buffer can be performed in an automated manner using an embodiment of the system 100 described above, prior to mixing the biological samples with magnetic beads in Block S410.

Step S420 recites heating the set of magnetic bead-sample mixtures to produce a set of nucleic acid-magnetic bead samples, and functions to incubate the set of magnetic bead-sample mixtures in order to lyse biological matter, and release nucleic acids to be bound to magnetic beads. Preferably, Step S420 comprises heating a capture plate containing the set of magnetic bead-sample mixtures for a specified amount of time at a specified temperature, and may additionally include cooling the set of magnetic bead-sample mixtures. In a specific example, Step S420 comprises heating a capture plate containing the set of magnetic beadsample mixtures using a capture plate module, wherein the capture plate module is configured to cradle and controllably heat wells containing the set of magnetic bead-sample mixtures. Step S420 may alternatively comprise incubating the set of magnetic bead-sample mixtures using any appro-

priate method and/or system as is known by those skilled in the art. Finally, Step S420 may be omitted in embodiments of the method 400 involving samples that do not require

heating.

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Step S430 recites transferring each nucleic acid-magnetic 5 bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways, and functions to isolate each of the set of nucleic acid-magnetic bead samples within separate pathways for further processing. Preferably, all nucleic acid-magnetic 10 bead samples in the set of nucleic acid-magnetic bead samples are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-magnetic bead sample in the set of magnetic bead-samples may be transferred to a corresponding fluidic pathway independently of 15 the other nucleic acid-magnetic bead samples. In addition, preferably the entire volume, or substantially all of the volume, of the nucleic acid-magnetic bead sample is transferred to the set of fluidic pathways, without magnetically isolating magnetic beads and removing supernatant fluids 20 prior to transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways.

Step S430 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of 25 a set of occlusion positions S432, which functions to define at least one truncated fluidic pathway. Preferably, Step S432 comprises defining at least one truncated fluidic pathway passing through at least one of a heating region and a magnetic field; however, Step S432 may alternatively not 30 comprise defining a truncated fluidic pathway passing through at least one of a heating region and a magnetic field.

In a specific example of Step S430, the multichannel liquid handling subsystem of Step S410 transfers a set of nucleic acid-magnetic bead samples to a set of fluidic 35 pathways of a microfluidic cartridge aligned within a molecular diagnostic module, wherein the microfluidic cartridge comprises an elastomeric layer in contact with the set of fluidic pathways. Manipulation of the elastomeric layer at a subset of a set of occlusion positions by a valve actuation 40 subsystem of the molecular diagnostic module defines a set of truncated fluidic pathways crossing a heating region and a magnetic field, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a truncated fluidic pathway of the set of 45 truncated fluidic pathways.

Step S440 recites producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples, and functions to separate nucleic acid volumes from the set of nucleic acid-magnetic bead samples. Step S440 preferably 50 reduces a concentration of unwanted matter from the set of biological samples being processed, to an acceptable level; however, Step S440 may alternatively entirely remove substantially all unwanted substances from the set of biological samples being processed. Step S440 preferably includes 55 providing a magnetic field S441, such that each fluidic pathway in the set of fluidic pathways is configured to cross the magnetic field. Preferably, the set of nucleic acidmagnetic bead samples is captured and isolated within portions of the set of fluidic pathways crossing the magnetic 60 field. Step S440 may further comprise providing a heater configured to span a heating region of the set of fluidic pathways S442, but may alternatively comprise providing multiple heaters or altogether omit providing a heater. In embodiments wherein multiple heaters are provided, each 65 heater is preferably independent to allow independent control of heating time and temperature for each sample. Step

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S442 functions to provide a heater, which, in combination with a release solution that provides a pH shift, facilitate a rapid and efficient unbinding of the nucleic acids from magnetic beads.

Step S440 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S443 (and opening a previously occluded channel), which functions to define at least one truncated fluidic pathway containing a nucleic acid-magnet bead sample and coupled to a source for delivery of a wash solution and a release solution. Preferably, Step S443 comprises defining at least one truncated fluidic pathway coupled to a waste chamber and to a fluid port, which functions to facilitate washing of at least one nucleic acidmagnetic bead sample in the set of nucleic acid-magnetic bead samples, and releasing of at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. Step S440 may additionally comprise delivering a wash solution through a portion of at least one fluidic pathway S444, such as the truncated fluidic pathway defined in Step S443, and delivering a release solution through a portion of at least one fluidic pathway S445, such as the truncated fluidic pathway defined in Step S443. Step S444 functions to wash at least one nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples, and Step S445 functions to release at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. The heater provided in Step S442 may be activated after Step S445 to induce a pH shift.

In a specific example of Step S440, the set of fluidic pathways containing a set of nucleic acid-magnetic bead samples, from the specific example of Step S430, is occluded at a subset of the set of occlusion positions by a valve actuation subsystem of the molecular diagnostic module, to define a set of truncated fluidic pathways coupled to a waste chamber and to a shared fluid port of the microfluidic cartridge for delivery of a wash solution and a release solution. The liquid handling system delivers a wash fluid through the shared fluid port to wash the set of nucleic acid-magnetic bead samples, captured within the magnetic field, and then delivers a release fluid through the shared fluid port to release a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples. In the specific example, each fluidic pathway is washed sequentially, and the release solution is delivered to each fluidic pathway sequentially to ensure that each lane is provided with substantially equal amounts of wash and release solutions. All waste fluid produced in the specific example of Step S440 pass into the waste chamber coupled to the set of truncated fluidic pathways.

Step S450 recites combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures, which functions to prepare the set of nucleic acid volumes to be detected. For each nucleic acid volume in the set of nucleic acid volumes, Step S450 preferably comprises aspirating an entire volume of the nucleic acid volume from its corresponding fluidic pathway, and transferring the nucleic acid volume to a well containing a molecular diagnostic reagent. Preferably, all nucleic acid volumes in the set of nucleic acid volumes are aspirated and combined with molecular diagnostic reagents simultaneously using a multichannel fluid delivery system; however, each nucleic acid volume in the set of nucleic acid volumes may alternatively be aspirated and combined with molecular diagnostic reagents independently of the other nucleic acid volumes. The molecular diagnostic reagents

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preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis 5 B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to detect any specific nucleic acid sequence.

In a first variation of Step S450 as shown in FIG. 16A, a 10 nucleic acid volume is aspirated and combined with a molecular diagnostic reagent for a single assay. In the first variation of Step S450, a set of nucleic acid volumes may thus be aspirated simultaneously, and each nucleic acid volume may be transferred to an individual well to be 15 combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures. In the first variation of Step S450, all nucleic acid-reagent mixtures in the set of nucleic acidreagent mixtures may or may not be substantially identical 20 in composition, depending on the homogeneity of the biological samples used in Step S410; however, the first variation of S450 preferably comprises using identical molecular diagnostic reagents, such that identical molecular diagnostic protocols analyzing identical markers may be performed. 25 Thus, the first variation of Step S450 encompasses running multiple identical tests from a stock biological sample (e.g., a multiplex assay), and running identical tests using a set of substantially different biological samples (e.g., from differ-

In a second variation of Step S450, as shown in FIG. 16B, the set of nucleic acid volumes is aspirated, and each nucleic acid volume in the set of nucleic acid volumes is combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents. In the second variation of Step S450, 35 the set of molecular diagnostic reagents preferably comprises different molecular diagnostic reagents, such that different molecular diagnostic protocols analyzing different markers may be performed. Thus, the second variation encompasses running multiple substantially different tests 40 using a stock biological sample, and running substantially different tests using substantially different biological samples (e.g., from different sources).

In a specific example of Step S450, a multichannel liquid handling system aspirates approximately 18 μL of each of a 45 set of nucleic acid volumes from the microfluidic cartridge used in the specific example of Step S440 using a set of pipette tips, punctures at least one foil seal 195 of at least one assay strip, wherein each well of the at least one assay strip contains molecular diagnostic reagents, and dispenses each 50 aspirated nucleic acid volume into a well of the assay strip. In the specific example of S450, the multichannel liquid handling system then aspirates and dispenses the contents of each well approximately 10 times to reconstitute molecular diagnostic reagents and mix the contents of each well.

Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated 60 detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acidreagent mixture in the set of nucleic acid reagent mixtures 65 may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures.

Step S460 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S462, which functions to define at least one truncated fluidic pathway coupled to a detection chamber of a set of detection chambers. Preferably, Step S462 comprises occluding each fluidic pathway of the set of

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fluidic pathways at a subset of a set of occlusion positions. thus defining a set of truncated fluidic pathways, each coupled to a detection chamber.

In a specific example of Step S460, the multichannel liquid handling subsystem of the specific example of Step S450 transfers a set of nucleic acid-reagent mixtures, each having a volume of approximately 16 µL, back to the set of fluidic pathways of the microfluidic cartridge of the specific example of Step S450. Each nucleic acid-reagent mixture in the set of nucleic acid-reagent mixtures is transferred at a rate of 50 μL/minute. Manipulation of the elastomeric layer at a subset of a set of occlusion positions by the valve actuation subsystem of the molecular diagnostic module defines a set of truncated fluidic pathways, each coupled to a detection chamber, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a truncated fluidic pathway of the set of truncated fluidic pathways. In the specific embodiment the occlusion position immediately upstream of the detection chamber and the occlusion position immediately downstream of the detection chamber are normally closed positions. During delivery, the multichannel liquid handling subsystem generates pressure to cause the elastomeric layer at the normally closed positions to deform and allow fluid to flow through the normally closed positions. Once the pressure drops after the detection chamber is filled and the multichannel liquid handing subsystem ceases delivery, the elastomeric layer is configured to overcome the pressure in the channel and recloses, thereby sealing the normally closed positions. The normally closed positions are then compressed using the valve actuation subsystem during thermocycling to prevent pressures generated during a molecular diagnostic assay to cause the normally closed positions to leak. After the molecular diagnostic assay is complete and the occlusion "pins" withdrawn, the normally closed positions allow the samples and amplicons to be trapped within detection chambers, substantially reducing the risk of contamination of the lab or other samples.

Step S470 recites receiving light from the set of nucleic acid-reagent mixtures, and functions to produce emission responses from the set of nucleic acid-reagent mixtures in response to transmission of excitation wavelength light or chemiluminescent effects. Preferably, Step S470 comprises the ability to transmit light including a wide range of wavelengths through a set of excitation filters and through a set of apertures configured to individually transmit light having single or multiple excitation wavelengths onto the set 55 of nucleic acid-reagent mixtures, and receiving light through a set of emission filters, from the set of nucleic acid-reagent mixtures. Step S470 may additionally comprise reflecting light from the set of excitation filters off of a set of dichroic mirrors, and transmitting light through the set of dichroic mirrors to a set of photodetectors. A specific example of Step S470 comprises using the optical subsystem 180 of the system 100 described above to transmit and receive light; however, alternative variations of Step S470 may use any appropriate optical system configured to transmit light at excitation wavelengths toward the set of nucleic acid-reagent mixtures, and to receive light at emission wavelengths from the set of nucleic acid-reagent mixtures.

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Step S480 recites generating a set of data based on light received from the set of nucleic acid-reagent mixtures, which functions to produce quantitative and/or qualitative data from the set of nucleic acid-reagent mixtures. Step S480 may further function to enable detection of a specific nucleic acid sequence from the nucleic acid-reagent mixture, in order to identify a specific nucleic acid sequence, gene, or organism. Preferably, Step S480 includes converting electrical signals, produced by a set of photodetectors upon receiving light from the set of nucleic acid-reagent mixtures, into a quantifiable metric; however, S480 may alternatively comprise converting electromagnetic energy, received by a set of photodetectors from the set of nucleic acid-reagent mixtures, into a set of qualitative data. In one variation of 15 Step S480, the set of data may be processed by a processor and rendered on a user interface; however, in other variations of Step S480, the set of data may alternatively not be rendered on a user interface.

The method 400 may further comprise re-running a 20 biological sample S490 if processing and/or analysis of the biological sample results in less than ideal results. Preferably, Step S490 occurs if an analysis of a biological sample is indeterminate due to machine or user error. Additionally, Step S490 preferably occurs automatically upon detection of 25 a less than ideal result, but may alternatively occur in response to a user prompt. Block S490 is enabled due to rapid processing enabled by an embodiment of the system 100 described above, wherein rerunning a sample without sample degradation is feasible during the time it takes to run 30 a biological sample and determine if an analysis of the biological sample has produced an indeterminate result.

Embodiments of the method 400 and variations thereof can be embodied and/or implemented at least in part by a storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system 100 and one or more portions of the processor 273 and/or the controller 272. The computer-readable medium can be stored on any suitable 40 computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computerexecutable component is preferably a general or application specific processor, but any suitable dedicated hardware or 45 hardware/firmware combination device can alternatively or additionally execute the instructions.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of systems, methods and computer program products according to preferred 50 embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should 55 also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse 60 order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be impleperform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

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As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following

#### We claim:

- 1. A system for processing and detecting nucleic acids in 10 cooperation with a cartridge comprising a heating region defined at a first surface, and a magnet housing region defined at a second surface in thermal communication with the first surface, the system comprising:
  - a capture plate configured to facilitate combination of a set of magnetic beads with a biological sample, thus producing a magnetic bead-sample; and
  - a molecular diagnostic module, configured to process the magnetic bead-sample from the capture plate and separate nucleic acids from the set of magnetic beads, comprising:
    - a cartridge platform configured to receive the cartridge and comprising a magnet receiving slot aligned with the second surface during operation,
    - a heater configured to transmit heat to the heating region at the first surface during operation, and
    - a magnet coupled to a magnet heating element that heats the magnet during operation with the magnet proximal the second surface, the magnet configured to pass through the magnet receiving slot into the magnet housing region during operation, thereby facilitating separation of a nucleic acid volume from the magnetic bead-sample and heating of the magnetic bead-sample in cooperation with the heater.
- 2. The system of claim 1, wherein the molecular diagmachine configured to receive a computer-readable medium 35 nostic module further comprises an actuator configured to displace the cartridge platform to pass the magnet through the magnet receiving slot into the magnet housing region and to position the heater at the heating region, wherein the actuator is coupled to the heater.
  - 3. The system of claim 2, wherein the heater is configured to heat a capture segment of the cartridge through the heating region from a first direction, and wherein the magnet is configured to apply a field to the capture segment between the first and second surfaces while heating the capture segment at the second surface during operation, through the magnet housing region, from a second direction substantially opposed to the first direction, wherein a first projection of the heating region onto a plane at least partially overlaps with a second projection of the magnet housing region onto the plane.
  - 4. The system of claim 3, wherein the capture segment is an s-shaped capture segment, and wherein in an operation mode of the system the magnet is configured to cross a subset of the capture segment in a direction perpendicular to a flow direction through the capture segment thereby providing a magnetic field at the subset of the capture segment to capture the magnetic bead-sample within the capture segment.
  - 5. The system of claim 3, wherein in an extended configuration of the actuator, the magnet is passed through the magnet receiving slot, and wherein in a retracted configuration of the actuator, the magnet is retracted from the magnet receiving slot.
- 6. The system of claim 2, wherein the actuator of the mented by special purpose hardware-based systems that 65 molecular diagnostic module is a linear actuator, configured to vertically displace the cartridge platform, and wherein the cartridge platform of the molecular diagnostic module is

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coupled to a set of springs configured to counteract a force provided by the linear actuator.

- 7. The system of claim 1, wherein the magnet is one of a set of magnets, each magnet in the set of magnets coupled to and separated from an adjacent magnet by one of a set of magnet heating elements.
- **8.** The system of claim **1**, further comprising a liquid handling system configured to transfer the magnetic bead-sample from the capture plate to the molecular diagnostic module, and to aspirate the nucleic acid volume from the molecular diagnostic module.
- **9**. The system of claim **8**, wherein the liquid handling system is further configured to automatically combine the biological sample with a buffer solution, couple to a filter, and deliver the biological sample with the buffer solution through the filter and into the capture plate.
- 10. The system of claim 1, wherein the molecular diagnostic module further comprises a valve actuation subsystem situated inferior to the cartridge platform including:
  - an actuation substrate having an array of active regions and an array of inactive regions, wherein each of the array of active regions is configured to provide a force in a displacement direction normal to a surface of the actuation substrate,
  - a set of pins, each pin in the set of pins including a distal end and a displacement region and operable in: a retracted configuration and an extended configuration, wherein in the retracted configuration an active region of the actuation substrate is aligned with the displacement region of the pin and the distal end of the pin is displaced in the displacement direction, and wherein in the extended configuration an inactive region of the actuation substrate is aligned with the displacement region of the pin and the distal end of the pin is displaced in a direction opposing the displacement direction and occludes the fluidic pathway at an occlusion position and

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- a valve actuator configured to provide relative displacement in a direction tangential to the displacement direction between the array of active regions and the set of pins.
- 11. The system of claim 1, wherein the molecular diagnostic module further comprises an optical subsystem comprising a first unit and a second unit, wherein each of the first unit and the second unit includes a set of excitation filters, a set of emission filters, a set of photodetectors aligned with the set of emission filters, and a set of dichroic mirrors configured to reflect light from the set of excitation filters toward one of a set of nucleic acid-reagent mixtures at the cartridge, and to transmit emitted light from one of the set of nucleic acid-reagent mixtures, through at least one of the set of emission filters, and toward at least one of the set of photodetectors.
- 12. The system of claim 11, wherein the molecular diagnostic module further includes a set of detection chamber heaters configured to heat a set of detection chambers through the second surface of the cartridge, and wherein the optical subsystem is configured to receive light, emitted from the set of nucleic acid-reagent mixtures at the set of detection chambers, from the first surface of the cartridge.
  - 13. The system of claim 12, wherein at least one of the set of detection chamber heaters includes a coating configured to mitigate reflection of light from the set of detection chamber heaters toward photodetectors of the optical subsystem.
  - 14. The system of claim 1, wherein the molecular diagnostic module is configured to receive and align a microfluidic cartridge comprising a set of sample port-reagent port pairs, a fluid port, a set of detection chambers, a waste chamber, an elastomeric layer, and a set of fluidic pathways, wherein each fluidic pathway of the set of fluidic pathways is coupled to a sample port-reagent port pair, the fluid port, and a detection chamber, comprises a capture segment configured to cross the magnet, and is configured to transfer a waste fluid to the waste chamber, and to be occluded upon deformation of the elastomeric layer.

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# EXHIBIT 31

# (12) United States Patent

Williams et al.

(10) Patent No.: US 9,738,887 B2 (45) Date of Patent: Aug. 22, 2017

#### (54) MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS

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#### (65) Prior Publication Data

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#### Related U.S. Application Data

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- (51) Int. Cl.

  B01L 3/00 (2006.01)

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(58) Field of Classification Search
CPC ...... B01L 7/52; B01L 3/527; B01L 3/502761;
B01L 9/00; B01L 3/567; B01L 3/508;
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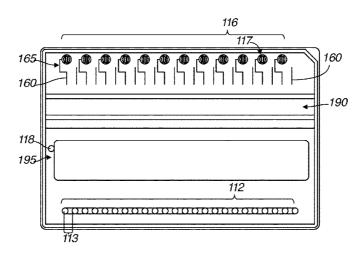
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Primary Examiner — Michael Hobbs Assistant Examiner — Liban Hassan (74) Attorney, Agent, or Firm — Jeffrey Schox; Ivan Wong

#### (57) ABSTRACT

A microfluidic cartridge, configured to facilitate processing and detection of nucleic acids, comprising: a top layer comprising a set of cartridge-aligning indentations, a set of sample port-reagent port pairs, a shared fluid port, a vent region, a heating region, and a set of Detection chambers; an intermediate substrate, coupled to the top layer comprising a waste chamber; an elastomeric layer, partially situated on the intermediate substrate; and a set of fluidic pathways, each formed by at least a portion of the top layer and a portion of the elastomeric layer, wherein each fluidic pathway is fluidically coupled to a sample port-reagent port pair, the shared fluid port, and a Detection chamber, comprises a turnabout portion passing through the heating region, and is configured to be occluded upon deformation of the elastomeric layer, to transfer a waste fluid to the waste chamber, and to pass through the vent region.

### 24 Claims, 18 Drawing Sheets



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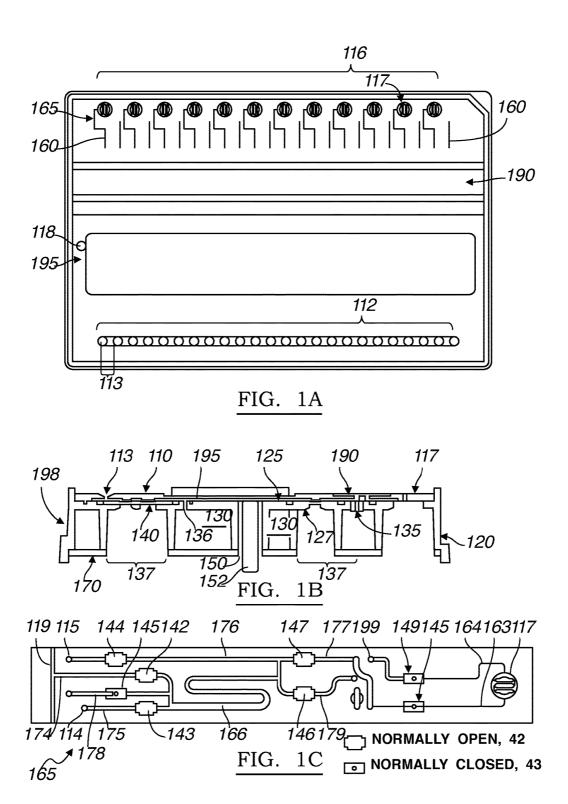
(51)	Int Cl				230	00/022; B01L 3/502746; B01L	
(31)	Int. Cl. C12Q 1/68 (2	006.01)				0861; B01L 2300/0887; B01L	
		006.01)				0/0481; B01L 2300/087; B01L	
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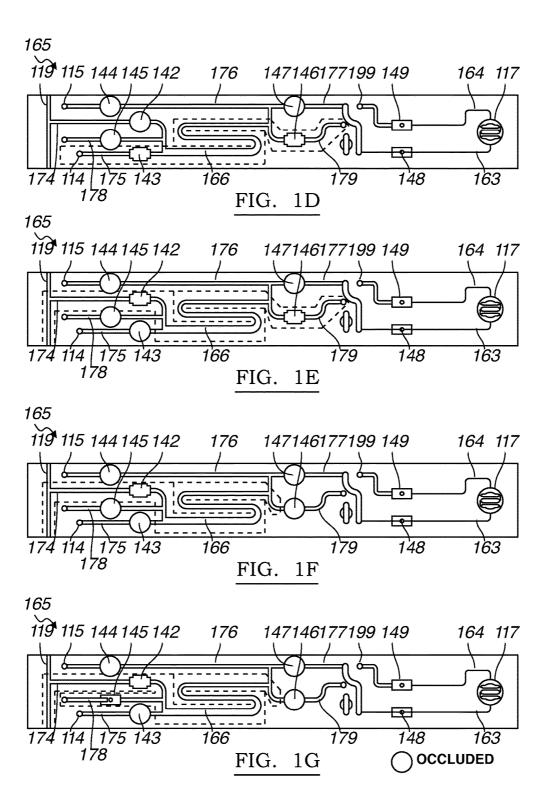
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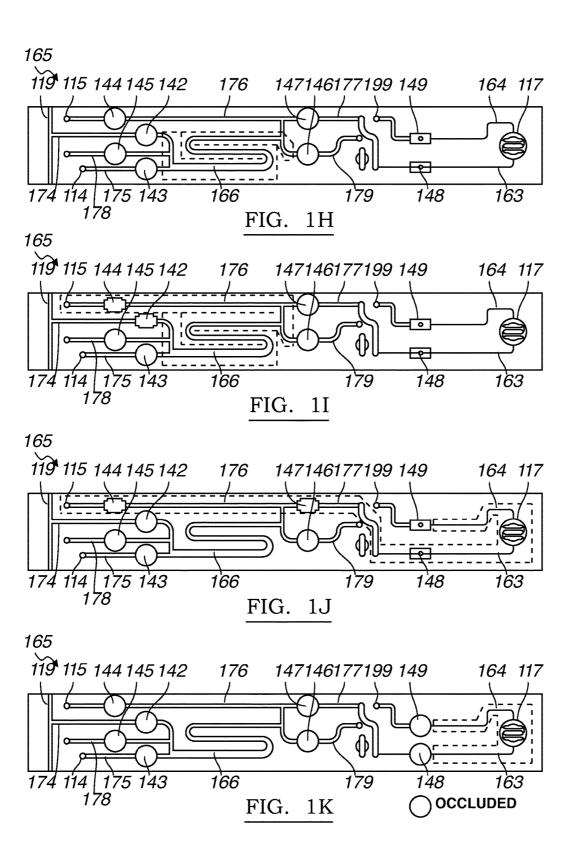
Aug. 22, 2017

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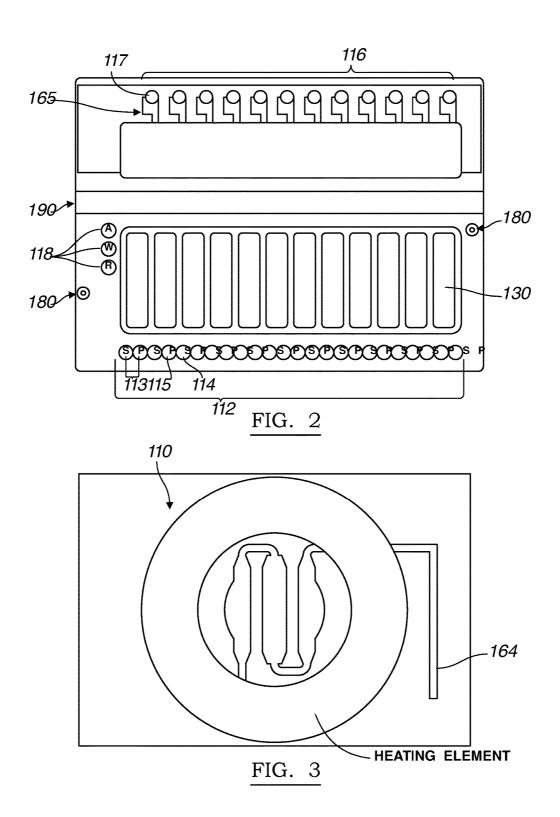
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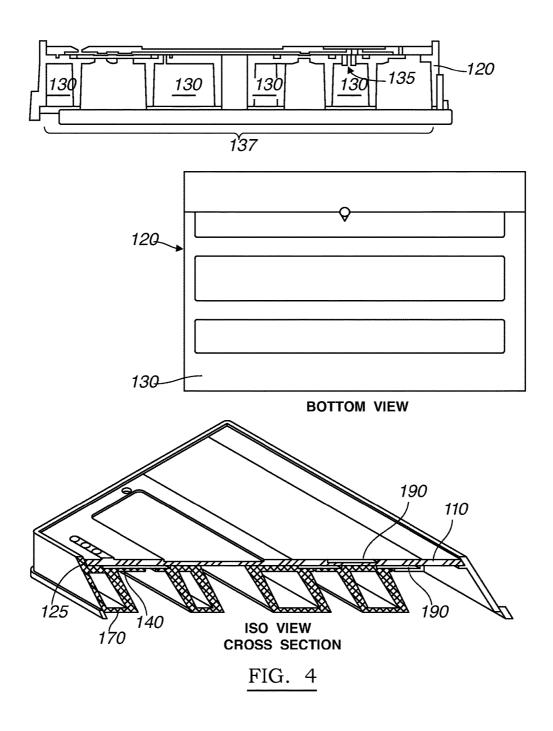
Aug. 22, 2017

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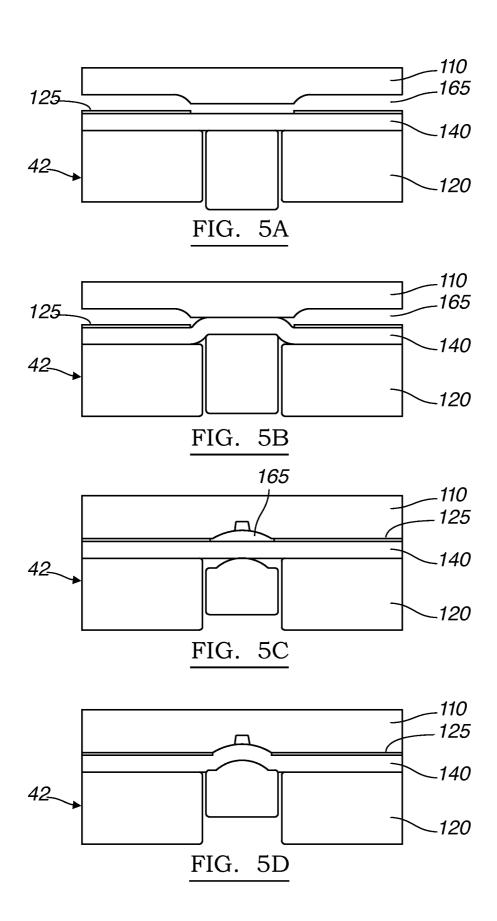
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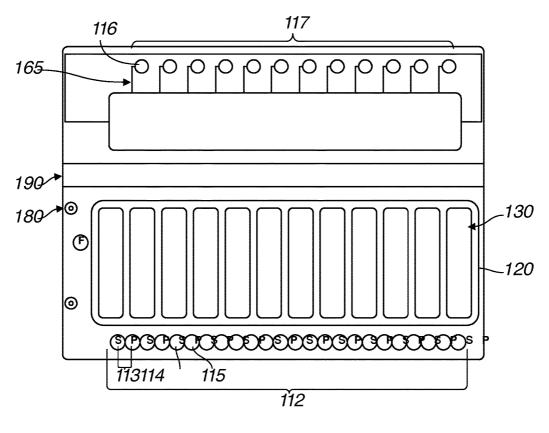
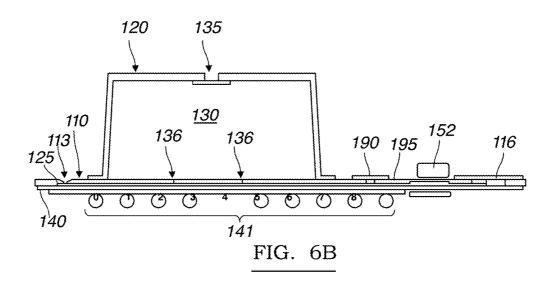


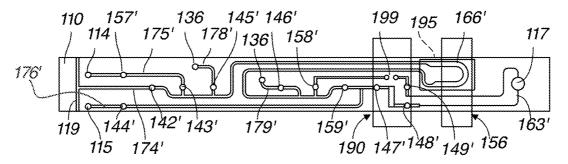
FIG. 6A

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The set of localusion positions (1411) encompasses elements 1421-1491 and 1571-1591

FIG. 6C

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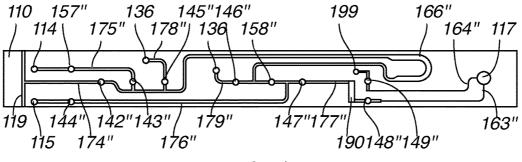
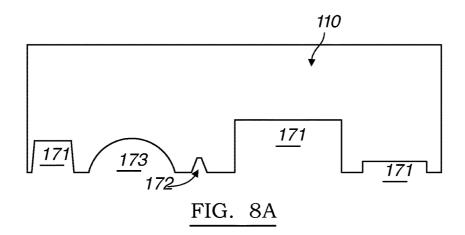


FIG. 7



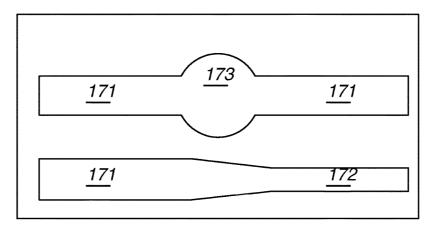
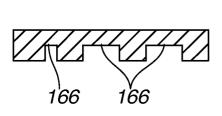
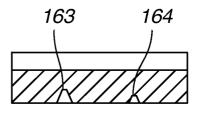


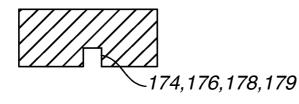
FIG. 8B

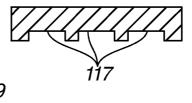
Aug. 22, 2017

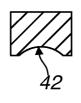
**Sheet 10 of 18** 

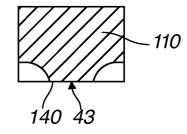












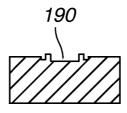
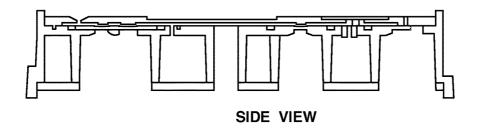


FIG. 8C

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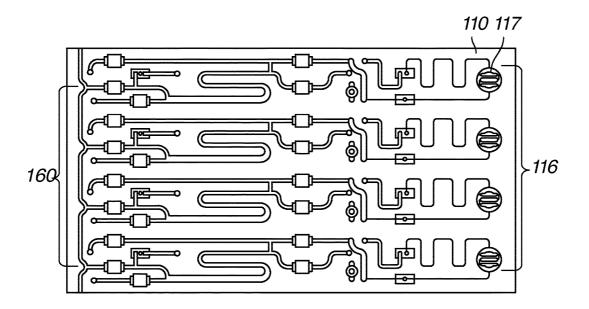


FIG. 9
TOP VIEW

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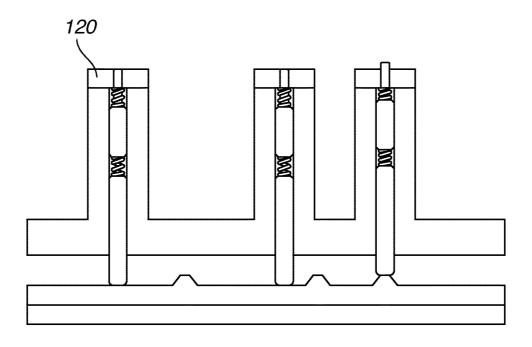


FIG. 10A

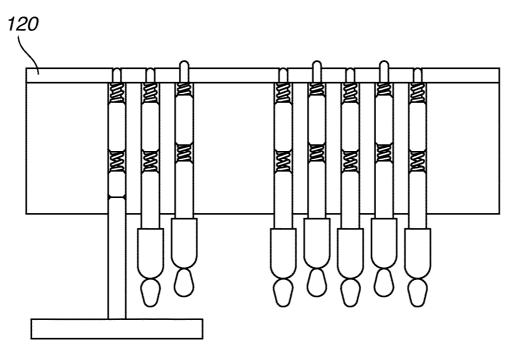
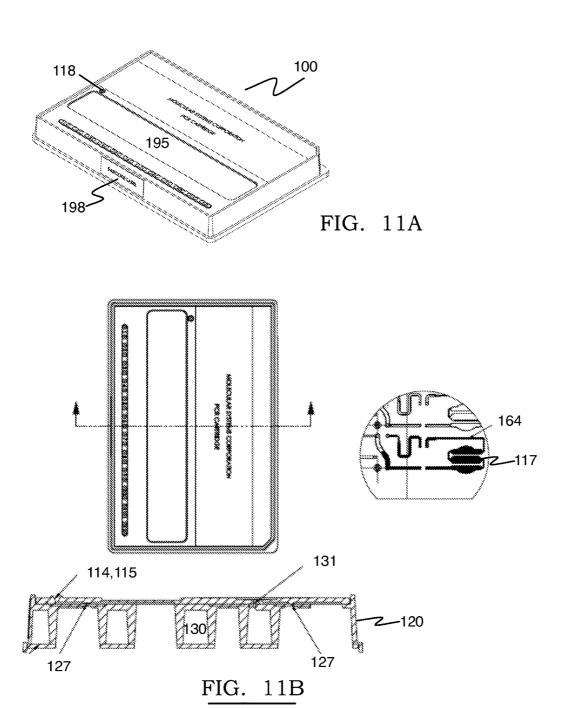


FIG. 10B

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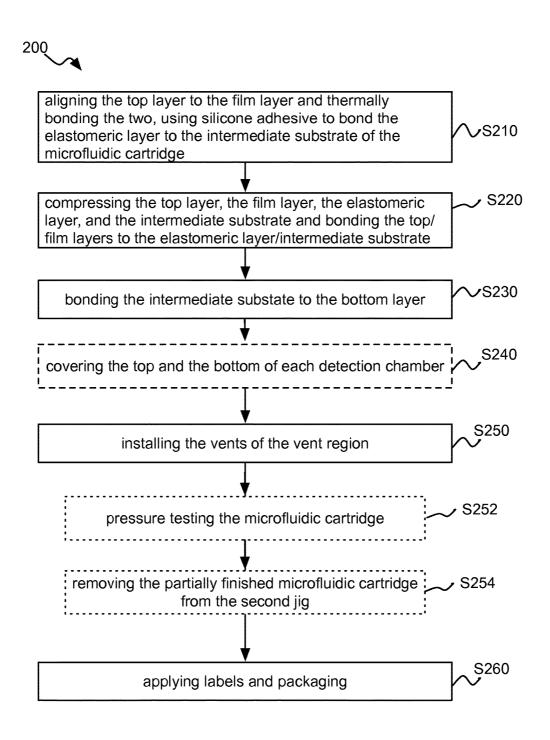
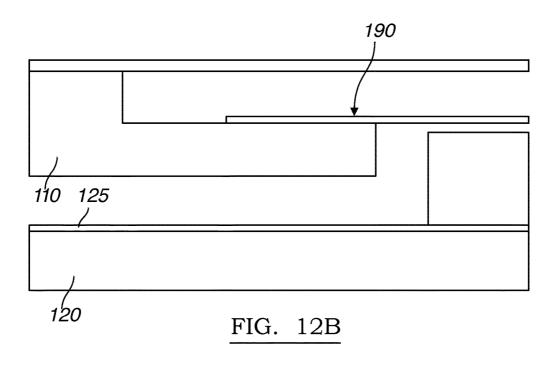


FIG. 12A

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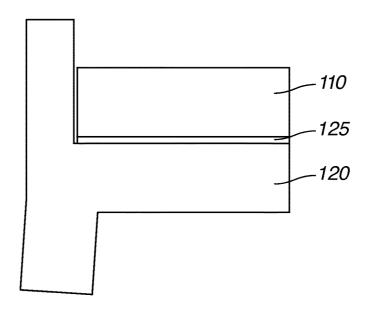
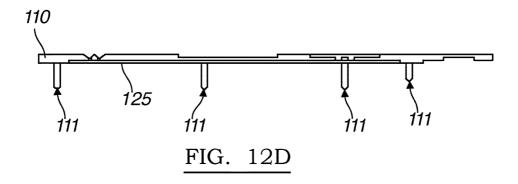
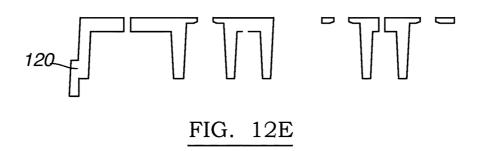


FIG. 12C

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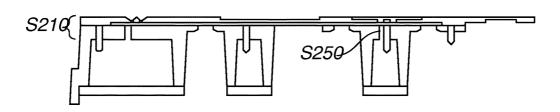


FIG. 12F

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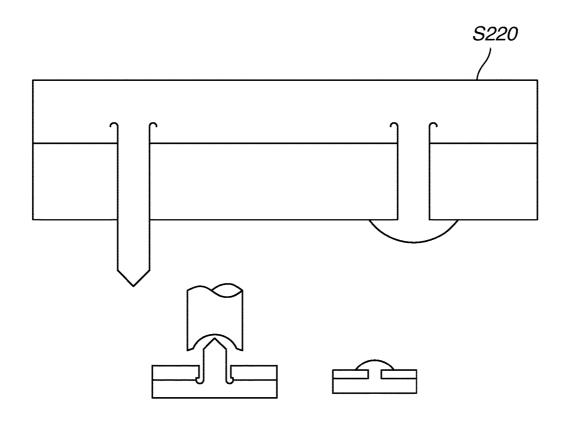


FIG. 12G

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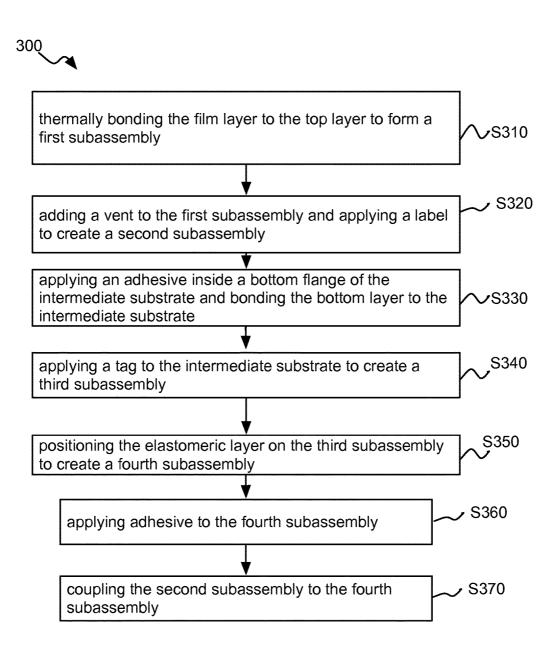


FIG. 13

#### 1

#### MICROFLUIDIC CARTRIDGE FOR PROCESSING AND DETECTING NUCLEIC ACIDS

# CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Ser. No. 61/667,606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on <sup>10</sup> 13 Feb. 2012, which are incorporated in their entirety by this reference

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved microfluidic cartridge for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology research procedures, but now has become an independent discipline 25 focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields requiring nucleic acid analysis. Molecular diagnostic analysis of biological samples can include the detection and/or monitoring 30 of one or more nucleic acid materials present in the specimen. The particular analysis performed may be either qualitative and/or quantitative. Methods of analysis may involve isolation, purification, and amplification of nucleic acid materials, and polymerase chain reaction (PCR) is a com- 35 mon technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and molecular diagnostic techniques are 40 also labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are often specific to certain nucleic acid types and not applicable across multiple acid types. Due to these and other deficien- 45 cies of current molecular diagnostic systems and methods, there is thus a need for improved devices for processing and amplifying nucleic acids. Thus, there is a need in the molecular diagnostics field to create an improved microfluidic cartridge to facilitate processing and detecting of 50 nucleic acids. This invention provides such a microfluidic cartridge.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1C depict an embodiment of a microfluidic cartridge (top and side views) and an embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. 1D-K depict an example embodiment of subsets of occlusion positions defining truncated portions of a fluidic 60 pathway;

FIG. 2 depicts an alternative embodiment of a microfluidic cartridge (top view) showing individual waste chambers located on the top of cartridge and multiple fluid ports;

FIG. 3 depicts an alternative embodiment of a detection 65 chamber of the microfluidic cartridge (top view) and a heating element configured to heat the detection chamber;

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FIG. 4 depicts an embodiment of a waste chamber of the microfluidic cartridge;

FIGS. 5A-5D depict embodiments of the elastomeric layer of the microfluidic cartridge, in open and occluded configurations;

FIGS. **6**A-**6**C depict an alternative embodiment of a microfluidic cartridge (top and side views) and an alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIG. 7 depicts another alternative embodiment of a microfluidic pathway of the microfluidic cartridge;

FIGS. **8**A and **8**B depict schematics of microfluidic channel cross sections;

FIG. **8**C depicts specific embodiments of microfluidic 15 channel cross sections;

FIG. 9 depicts an embodiment of the microfluidic cartridge with twelve fluidic pathways (four of which are shown):

FIGS. 10A and 10B depict embodiments of occlusion of 20 fluidic pathways with the elastomeric layer and a valving mechanism:

FIGS. 11A and 11B depict an embodiment of the microfluidic cartridge;

FIGS. 12A-12G depict an example manufacturing method for an embodiment of the microfluidic cartridge; and

FIG. 13 depicts an alternative example manufacturing method for an embodiment of the microfluidic cartridge.

# DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

1. Microfluidic Cartridge

As shown in FIGS. 1A-1C, an embodiment of a microfluidic cartridge 100 for processing and detecting nucleic acids comprises: a top layer 110 comprising a set of sample port-reagent port pairs 112 and a set of detection chambers 116; an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer by a film layer 125, configured to form a waste chamber 130; an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; and a set of fluidic pathways 160, each formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140. In other embodiments, the microfluidic cartridge 100 may further comprise a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber 130. Furthermore, the top layer 110 of the microfluidic cartridge 100 may further comprise a shared fluid port 118, a vent region 190, and a 55 heating region 195, such that each fluidic pathway 165 in the set of fluidic pathways 160 is fluidically coupled to a sample port-reagent port pair 113, the shared fluid port 118, the waste chamber 130, and a detection chamber 117, comprises a capture segment 166 configured to pass through the heating region and the magnetic field, and is configured to pass through the vent region 190 upstream of the detection chamber 117. Each fluidic pathway 165 thus functions to receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 165. As configured, the microfluidic cartridge 100 can be used to facilitate molecular diagnostic

processes and techniques, and preferably conforms to micro-

titer plate dimensional standards. Alternatively, the microfluidic cartridge 100 may be any appropriate size. In a specific application, the microfluidic cartridge 100 can be used to facilitate a PCR procedure for analysis of a sample

# 1.1 Microfluidic Cartridge—Top Layer

containing nucleic acids.

The top layer 110 of an embodiment of the microfluidic cartridge 100 functions to accommodate elements involved in performing a molecular diagnostic procedure (e.g. PCR), such that a sample containing nucleic acids, passing through 10 the cartridge, can be manipulated by the elements involved in performing the molecular diagnostic procedure. The top layer 110 is preferably composed of a structurally rigid/stiff material with low autofluorescence, such that the top layer 110 does not interfere with sample detection by fluorescence 15 or chemiluminescence techniques, and an appropriate glass transition temperature and chemical compatibility for PCR or other amplification techniques. Preferably, the top layer 110 is composed of a polypropylene-based polymer, but the top layer 110 may alternatively be composed of any appro- 20 priate material (e.g. cyclic olefin polymer). In a specific embodiment, the top layer 110 is composed of 1.5 mm thick polypropylene produced by injection molding, with a glass transition temperature between 136 and 163° C. The top layer 110 may alternatively be composed of any appropriate 25 material, for example, a polypropylene based polymer. As shown in FIGS. 1B and 1C, the top layer 110 preferably comprises a set of sample port-reagent port pairs 112, a fluid port 118, a vent region 190, a heating region 195 crossing a capture segment 166 of a fluidic pathway 165, and a set of 30 detection chambers 116.

Each sample-port-reagent port pair 113 of an embodiment of the top layer no comprises a sample port 114 and a reagent port 115. The sample port 114 functions to receive a volume of a sample fluid potentially containing the nucleic acids of 35 interest for delivery of the volume of fluid to a portion of a fluidic pathway 165 coupled to the sample port-reagent port pair 113. In a specific embodiment, the volume of a sample fluid is a biological sample with magnetic beads for nucleic acid isolation; however, the volume of fluid comprising a 40 sample fluid may alternatively be any appropriate fluid containing a sample with nucleic acids. Preferably, each sample port 114 is isolated from all other sample ports, in order to prevent cross-contamination between samples of nucleic acids being analyzed. Additionally, each sample port 45 114 is preferably of an appropriate geometric size and shape to accommodate a standard-size pipette tip used to deliver the volume of a sample fluid without leaking. Alternatively, all or a portion of the sample ports 114 are configured to be coupled to fluid conduits or tubing that deliver the volume 50 of a sample fluid.

Each sample-port reagent port pair 113 of an embodiment of the top layer 110 also comprises a reagent port 115, as shown in FIG. 1A. The reagent port 115 in a sample port-reagent port pair 113 functions to receive a volume of 55 fluid comprising a reagent used in molecular diagnostics, for delivery of the volume of fluid comprising a reagent to a portion of a fluidic pathway 165 coupled to the sample port-reagent port pair 113. In a specific embodiment, the volume of fluid comprising a reagent used in molecular 60 diagnostics is a sample of reconstituted molecular diagnostic reagents mixed with nucleic acids released and isolated using the microfluidic cartridge 100; however, the volume of fluid comprising a reagent used in molecular diagnostics may alternatively be any appropriate fluid comprising 65 reagents used in molecular diagnostics. Preferably, each reagent port 115 is isolated from all other reagent ports, in

order to prevent cross-contamination between samples of nucleic acids being analyzed. Additionally, each reagent port 115 is preferably of an appropriate geometric size to accommodate a standard-size pipette tip used to deliver the volume of fluid comprising a reagent used in molecular diagnostics.

Alternatively, all or a portion of the reagent ports 115 are configured to be coupled to fluid conduits or tubing that deliver the volume of fluid comprising a reagent used in molecular diagnostics.

Preferably, the set of sample port-reagent port pairs 112 is located near a first edge of the top layer 110, such that the configuration of the sample port-reagent port pairs 112 functions to increase accessibility, for instance, by a pipettor delivering fluids to the microfluidic cartridge 100. In one specific example, the microfluidic cartridge 100 is configured to be aligned within a module, with the set of sample port-reagent port pairs 112 accessible outside of the module, such that a multichannel pipette head can easily access the set of sample port-reagent port pairs 112. Preferably, as shown in FIG. 1A, the set of sample port-reagent port pairs 112 is configured such that the sample ports 114 and the reagent ports 115 alternate along the first edge of the top layer 110. In an alternative embodiment, the set of sample port-reagent port pairs 112 may not be located near an edge of the top layer 110, and may further not be arranged in an alternating fashion.

The fluid port 118 of the top layer 110 of the microfluidic cartridge functions to receive at least one of a wash fluid, a release fluid, and a gas used in a molecular diagnostic procedure, such as PCR. In an embodiment, the wash fluid, the release fluid, and/or the gas are common to all samples being analyzed during a run of the diagnostic procedure using the microfluidic cartridge 100; in this embodiment, as shown in FIG. 1A, the fluid port 118 is preferably a shared fluid port, fluidically coupled to all fluidic pathways 165 coupled to the sample port-reagent port pairs 112, and configured to deliver the same wash fluid, release fluid, and/or gas through the shared fluid port. Alternatively, as shown in FIG. 2, the top layer may comprise more than one fluid port 118, configured to deliver different wash fluids, release fluids, and/or gases to individual or multiple fluidic pathways 165 coupled to the set of sample port-reagent port pairs 112.

Preferably, the fluid port 118 is located along an edge of the microfluidic cartridge 100, which functions to increase accessibility to the fluid port by a system delivering fluids to the fluid port 118. In a specific embodiment, as shown in FIG. 1A, the fluid port is located approximately midway along an edge of the microfluidic cartridge 100, different from the edge along which the set of sample port-reagent port pairs 112 is located. Alternatively, the fluid port 118 may not be located along an edge of the microfluidic cartridge 100. Additionally, the fluid port 118 is preferably configured to be coupled to a syringe pump for fluid delivery; however, the fluid port 118 may alternatively configured to couple to any appropriate system for fluid delivery. Preferably, the wash fluid is a wash buffer for washing bound nucleic acid samples (i.e. nucleic acids bound to magnetic beads), the release fluid is a reagent for releasing bound nucleic acids samples from the magnetic beads, and the gas is pressurized air for moving fluids and demarcating separate reagents. Alternatively, the wash fluid, release fluid, and gas may be any appropriate liquids or gases used to carry out a molecular diagnostic procedure.

The heating region 195 of the top layer 110 functions to accommodate and position a heating element relative to elements of the microfluidic cartridge 100. The heating

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element preferably heats a defined volume of fluid and the magnetic beads, which has traveled through the microfluidic cartridge 100, according to a specific molecular diagnostic procedure protocol (e.g. PCR protocol), and is preferably an element external to the microfluidic cartridge 100; alterna- 5 tively, the heating element may be integrated with the microfluidic cartridge and/or comprise a thermally conductive element integrated into the microfluidic cartridge 100. The heating region 195 is preferably a recessed fixed region of the top layer 110, downstream of the sample port-reagent 10 port pairs 112, as shown in FIGS. 1A and 1B. Alternatively, the heating region may not be fixed and/or recessed, such that the heating region 195 sweeps across the top layer 110 of the microfluidic cartridge 100 as the heating element is moved. The microfluidic cartridge 100 may altogether omit 15 the heating region 195 of the top layer 110, in alternative embodiments using alternative processes (e.g. chemical methods) for releasing nucleic acids from nucleic acidbound magnetic beads.

The vent region 190 of an embodiment of the top layer 20 110 functions to remove unwanted gases trapped within a fluidic pathway 165 of the microfluidic cartridge, and may additionally function to position a defined volume of fluid within a fluidic pathway 165 of the microfluidic cartridge. The vent region 190 is preferably located downstream of the 25 heating region 195 in an embodiment where the heating region 195 is fixed on the top layer 110 of the microfluidic cartridge 100, but alternatively may be located at another appropriate position on the top layer 110 such that unwanted gases are substantially removed from the microfluidic car- 30 tridge 100 during analysis. The top layer 110 may alternatively comprise more than one vent region 190 located at appropriate positions in the top layer 110. Preferably, as shown in FIGS. 1A and 1B, the vent region 190 is a recessed region in the top layer 110, and further comprises a film 35 covering the vent region 190. Preferably, the film covering the vent region 190 is a gas-permeable but liquid-impermeable film, such that unwanted gases may be released from the microfluidic cartridge 100, but fluids remain within the microfluidic cartridge 100 and flow to the point of contact- 40 ing the film. This functions to remove unwanted gases and position a defined volume of fluid within a fluidic pathway 165 of the microfluidic cartridge. In a specific embodiment, the film covering the vent region is a hydrophobic porous polytetrafluoroethylene-based material, synthesized to be 45 gas-permeable but liquid-impermeable. Alternatively, the film covering the vent region may be gas and liquid permeable, such that unwanted gases and liquids are expelled from the microfluidic cartridge 100 through the vent region 190. Other alternative embodiments of the microfluidic cartridge 50 100 may altogether omit the vent region.

The set of detection chambers 116 of an embodiment of the top layer 110 functions to receive a processed nucleic acid sample, mixed with molecular diagnostic reagents, for molecular diagnostic analysis. Preferably, the set of detec- 55 tion chambers 116 is located along an edge of the top layer 110, opposite the edge along which the set of sample port-reagent port pairs 112 is located, which allows sample fluids dispensed into the microfluidic cartridge 100 to be processed and mixed with molecular diagnostic reagents on 60 their way to a detection chamber 117 of the set of detection chambers 116 and facilitates access to the detection chambers by external elements performing portions of a molecular diagnostics protocol (e.g. heating and optics systems). Alternatively, the set of detection chambers 116 may not be 65 located along an edge of the top layer 110. In a first variation, as shown in FIGS. 1A and 11B, each detection chamber 117

in the set of detection chambers comprises a serpentineshaped channel 16 for facilitating analysis of a solution of nucleic acids mixed with reagents. In the first variation, three portions of the serpentine-shaped channel 16 are preferably wide and shallow to facilitate heating, and are interconnected by two narrow portions, which function to increase fluid flow resistance and reduce the proportion of nucleic acid not contained within the detection area. The first variation functions to facilitate filling of the set of detection chambers in a manner that reduces the potential for trapped air bubbles, to facilitate rapid molecular diagnostic techniques, and to comply with current imaging technologies. In a specific example of the first variation, each serpentineshaped channel 16 is injected molded into the top layer 110 of the microfluidic cartridge 100, and the three interconnected portions of the serpentine-shaped channel 16 are each  $1600 \mu m$  wide by  $400 \mu m$  deep.

In a second variation, each detection chamber 117 in the set of detection chambers has a depth between 0.400 mm and 1.00 mm, and a diameter between 3.50 mm and 5.70 mm, to provide a volumetric configuration that facilitates reaction efficiency. In a specific example of the second variation, each detection chamber 117 in the set of detection chambers 116 is configured to contain a total volume of 10 uL, and has a depth of 0.80 mm and a diameter of 3.99 mm; however, in alternative embodiments, each detection chamber 117 in the set of detection chambers 116 may be configured to contain a total volume less than or greater than 10 uL.

Preferably, as shown in FIGS. 1A and 1B, the lower regions of each detection chamber 117 in the set of detection chambers 116 includes a PCR compatible film that is thin, to facilitate efficient thermocycling, and has low autofluorescence, to facilitate light-based molecular diagnostic assays performed at the set of detection chambers 116. The PCR compatible film is preferably composed of a polypropylene based polymer thermally bonded to the bottom of the top layer, but may alternatively be composed of any appropriate PCR-compatible material and bonded in any fashion. In one specific variation, the PCR compatible film is a cyclic olefin polymer (COP) film, thermally bonded to the top layer 110, with a glass transition temperature suitable for a molecular diagnostic protocol. In one alternative embodiment, depending on the configuration of imaging, heating, and/or cooling elements external to the microfluidic cartridge 100, the top and/or bottom of the detection chambers 117 in the set of detection chambers 116 may be entirely formed of a clear or transparent material (e.g. glass or plastic) allowing transmission of light. In a variation of this alternative embodiment, lensing, other optical components, or additional structures may also be incorporated into the detection chambers, to facilitate light transmission and/or focusing. In the variation of the alternative embodiment, a lens may be manufactured (e.g. injection molded) directly to form a surface of a detection chamber 117.

In the embodiment of the set of detection chambers 116 that includes a PCR compatible film, the PCR compatible film may further include a thermally conductive component, which functions to transfer heat from a heating element to the detection chamber. Depending on the position of the heating element(s) relative to the microfluidic cartridge 100 during analysis, the thermally conductive component of the PCR compatible film may be integrated with just the upper region of each detection chamber, just the lower region of each detection chamber. The thermally conductive component of the PCR compatible film may comprise a wire

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mesh with a substantially small wire diameter, as shown in FIG. 3, thermally conductive particles distributed through the PCR compatible film (in a manner that still allows for optical clarity), or any other appropriate thermally conductive component (e.g. thermally conductive beads integrated 5 into the PCR compatible film). The region laterally around the detection chamber may also further include one or more heat-transfer elements or air channels speed heat dissipation. Alternatively, a detection chamber 117 in the set of detection chambers 116 may not include a PCR compatible film with 10 a thermally conductive component. Preferably, each detection chamber 117 is heated using a diced silicon wafer with conductive channels flip-chip bonded to a detection chamber to provide resistive heating; however, each detection chamber 117 may alternatively be heated using any appropriate 15 heating device or method, and may be assembled using any appropriate method.

Preferably, each detection chamber 117 in the set of detection chambers 116 is thermally isolated from all other detection chambers, in order to prevent contamination of 20 data from a detection chamber 117 due to heat transfer from other detection chambers in the set of detection chambers 116. In one embodiment, each detection chamber 117 of the set of detection chambers 116 is spaced far from adjacent detection chambers to limit thermal crosstalk. In another 25 alternative embodiment, the top layer 110 may comprises slots between adjacent detection chambers to separate the detection chambers with an air gap. In one variation, thermal isolation is achieved by surrounding the side walls of each detection chamber 117 with a thermally insulating material, 30 such as an insulating epoxy, putty, filler, or sealant. In another variation, the thermally insulating material has a low density, which functions to reduce heat transfer from other detection chambers. In yet another variation, thermal isolation is achieved by geometrically separating or displacing 35 the detection chambers relative to each other within the top layer 110 of the microfluidic cartridge 100, such that heat transfer between detection chambers is hindered.

Preferably, each detection chamber 117 in the set of detection chambers 116 is also optically isolated from all 40 other detection chambers, in order to prevent contamination of data from a detection chamber 117 due to light transfer from other detection chambers in the set of detection chambers 116. Preferably, optical isolation is achieved with detection chambers having substantially vertical walls, and 45 separating each detection chamber 117 in the set of detection chambers from each other. However, in one variation, the sidewalls of each detection chamber 117 in the set of detection chambers 116 are either composed of or surrounded by a material with low autofluorescence and/or poor 50 optical transmission properties to achieve optical isolation. In another variation, the sidewalls of each detection chamber 117 are surrounded by an optically opaque material, thus allowing transmission of light to a detection chamber 117 through only the top and bottom regions of the detection 55 chamber 117. Alternatively, the microfluidic cartridge 100 may not further comprise any provisions for optical isolation of each detection chamber 117 in the set of detection chambers 116, aside from constructing the set of detection chambers 116 with a material having low autofluorescence. 60

Additionally, each detection chamber 117 in the set of detection chambers 116 may be further optimized to meet volumetric capacity requirements, facilitate high thermocycling rates, facilitate optical detection, and facilitate filling in a manner that limits bubble generation. Alternatively each 65 detection chamber 117 in the set of detection chambers 116 may not be optimized to meet volumetric capacity require-

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ments, facilitate high thermocycling rates, facilitate optical detection, and/or facilitate filling in a manner that limits bubble generation.

The top layer 110 of the microfluidic cartridge 100 may further comprise a set of cartridge-aligning indentations 180, which function to align the microfluidic cartridge 100 as it moves through an external module. As shown in FIG. 2 the set of cartridge-aligning indentations 180 are preferably located such that they do not interfere with any ports 112, 118, the heating region, 195, the vent region 190, and/or the set of detection chambers 116. In an embodiment, the top layer 110 of the microfluidic cartridge preferably comprises at least four cartridge-aligning indentations, located at points on the periphery of the top layer 110, and the cartridgealigning indentations are configured to be recessed regions configured to mate with alignment pins in a system external to the microfluidic cartridge 100. Alternatively, the cartridge-aligning indentations may be grooves, such that the microfluidic cartridge 100 accurately slides into position along the grooves within a system external to the microfluidic cartridge 100. In yet another alternative embodiment, the set of cartridge-aligning indentations 180 may be any appropriate indentations that allow for positioning of the microfluidic cartridge 100 within an external system. However, the microfluidic cartridge 100 may altogether omit the set of cartridge-aligning indentations 180, and rely upon other features of the microfluidic cartridge 100 to facilitate alignment.

#### 1.2 Microfluidic Cartridge—Intermediate Substrate

As shown in FIG. 1B, an embodiment of the microfluidic cartridge also comprises an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer 110 by a film layer 125, configured to form a waste chamber 130. The intermediate substrate 120 functions to serve as a substrate to which layers of the microfluidic cartridge may be bonded, to provide guides for the valve pins, and to provide a waste chamber volume into which a waste fluid may be deposited. Preferably, the depth of the intermediate substrate 120 provides a waste chamber volume adequate to accommodate the volume of waste fluids generated within the microfluidic cartridge 100. Additionally, the depth of the intermediate substrate 120 provides a low profile for the microfluidic cartridge 100 to facilitate movement throughout a compact molecular diagnostic system. Preferably, the intermediate substrate 120 of the microfluidic cartridge 100 is also configured such that the footprint of microfluidic cartridge 100 adheres to microtiter plate standards, to facilitate automated handling of the microfluidic cartridge 100. The intermediate substrate 120 is preferably composed of a low-cost, structurally stiff material, such as polypropylene. However, similar to the top layer 120, the intermediate substrate may be alternatively composed of a structurally stiff material with low autofluorescence, such that the intermediate substrate 120 does not interfere with sample detection by fluorescence techniques, and an appropriate glass transition temperature for PCR techniques. In one variation of this alternative embodiment, the intermediate substrate 120 is composed of a cyclic olefin polymer (COP), produced by injection molding, with a glass transition temperature between 136 and 163° C. In yet another alternative embodiment, the intermediate substrate 120 may be composed of any appropriate material, for example, a polycarbonate based polymer.

Preferably, the intermediate substrate 120 of the microfluidic cartridge 100 is coupled to the top layer 110 and partially separated from the top layer 110 by a film layer 125. The film layer 125 functions to isolate individual fluidic

pathways 165 of the microfluidic cartridge, to prevent leakage, to provide an appropriate environment for sample processing and conducting a molecular diagnostic protocol, and to provide access between a microfluidic channel (of a fluidic pathway 165) above the film layer 125 and elements 5 below the film layer 125 (e.g. waste chamber and/or fluidic pathway occluder). Preferably, the film layer is a polypropylene (PP) with an appropriate glass transition temperature, such that it is PCR compatible and thermally bondable to the top layer 110; however, the film layer may alternatively be any appropriate material. In a specific embodiment, the film layer 125 is a polypropylene film between 30 and 100 microns thick and die cut to produce openings at a set of occlusion positions, to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer 15 125 and elements below the film layer 125. In this specific embodiment, the openings are slightly oversized prior to assembly, in order to allow for constriction during assembly (due to thermal and pressure effects) and to provide higher tolerance during assembly of microfluidic cartridge layers. 20 Alternatively, the film layer is any appropriate material such that it substantially isolates individual fluidic pathways, and is easily processable to provide access between a microfluidic channel of a fluidic pathway 165 above the film layer and elements below the film layer 125.

Preferably, the top layer 110, the film layer 125, and the intermediate substrate are bonded together, such that the top layer 110, film layer, 125, and intermediate substrate form a bonded unit with a hermetic seal to prevent fluid leakage. A hermetic seal is preferably formed using a silicone rubber 30 layer coupled to the film layer 125, but may alternatively be formed using an alternative material or method. In a specific embodiment, a hermetic seal formed using a silicone rubber layer is only required at locations of openings within the film layer (e.g, at locations where an external occluder interacts 35 with the microfluidic cartridge). Preferably, in an embodiment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially identical materials (e.g. polypropylene), at least one of thermal bonding, adhesives, and ultrasonic welding are used to coupled the 40 layers 110, 125, 120 together. In an embodiment where the top layer 110, the film layer 125, and the intermediate substrate 120 are substantially different materials—a combination of thermal bonding methods and adhesives may be used to bond the top layer 110, the film layer 125, and the 45 intermediate substrate 120 of the microfluidic cartridge 100 together. In an alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 of the microfluidic cartridge 100 may be thermally bonded together in a single step. In yet another alternative embodi- 50 ment, the top layer 110, the film layer 125, and the intermediate substrate 120 may alternatively be modular, in applications where a portion of the microfluidic cartridge 100 is partially reusable (e.g. in an application where the waste chamber may be discarded after use, but the top layer 55 and film may be reused). In yet another alternative embodiment, the top layer 110, the film layer 125, and the intermediate substrate 120 may only be partially bonded, such that a molecular diagnostic system, into which the microfluidic cartridge 100 is loaded, is configured to compress the 60 top layer 110, the film layer 125, and the intermediate substrate 120 together, preventing any fluid leakage.

As shown in FIG. 1B, the intermediate substrate 120 of an embodiment of the microfluidic cartridge 100 is configured to form a waste chamber 130, which functions to receive and 65 isolate waste fluids generated within the microfluidic cartridge 100. The waste chamber 130 is preferably continuous

fluidic cartridge 100, such that all waste fluids generated within the microfluidic cartridge 100 are deposited into a common waste chamber; however, each fluidic pathway 165 of the microfluidic cartridge 100 may alternatively have its own corresponding waste chamber 130, such that waste fluids generated within a fluidic pathway 165 of the microfluidic cartridge 100 are isolated from waste fluids generated within other fluidic pathways 165 of the microfluidic cartridge 100. In a specific embodiment of the microfluidic cartridge 100 with a continuous waste chamber, the waste chamber has a volumetric capacity of approximately 25 mL; however, the waste chamber 130 of another embodiment may have a different volumetric capacity. The intermediate substrate 120 further comprises a waste vent 135, which provides access between a microfluidic channel of a fluidic pathway 165 above the film layer 125 and the waste chamber 130. Preferably, the intermediate substrate 130 comprises more than one waste inlet 136, such that the waste chamber

is accessible at more than one location along a fluidic

pathway 165 through the waste inlets 136. Alternatively, the

intermediate substrate 120 may include a single waste inlet 136, such that all waste fluids generated within the micro-

fluidic cartridge 100 are configured to travel through the

single waste inlet 136 into the waste chamber 130. Also, as

shown in FIG. 1B, the intermediate substrate 120 may

comprise a waste vent 131, such that the waste chamber 130

is vented to prevent pressure build up in the waste chamber

as waste fluid is added.

As shown in FIGS. 1B and 4, the waste chamber 130 formed by the intermediate substrate 120 preferably has a corrugated surface 137, such that the waste chamber 130 is not only configured to receive and isolate a waste fluid, but also functions to 1) provide structural stability for the microfluidic cartridge 100 and 2) allow elements external to the microfluidic cartridge 100 to enter spaces formed by the corrugated surface 137, for greater accessibility to elements of the microfluidic cartridge 100. Also shown in FIGS. 1B and 4, each of the ridges in the corrugated surface 137 may not have the same dimensions, as a result of the locations of elements within and external to the microfluidic cartridge 100. In an embodiment of the waste chamber 130 with a corrugated surface 137, at least two ridges of the corrugated surface 137 are preferably the same height, such that the microfluidic cartridge 100 sits substantially level on a flat base. In an alternative embodiment, all ridges of the corrugated surface 137 of the waste chamber 130 are identical, for structural symmetry, and in yet another embodiment, the waste chamber 130 may not have a corrugated surface 137.

In one preferred embodiment, the intermediate substrate 120 of the microfluidic cartridge 100 further comprises a set of valve guides, which function to direct a series of external pins or other indenters through the valve guides at a set of occlusion positions 141, thus affecting flow through a microfluidic channel of a fluidic pathway 165 at the set of occlusion positions 141. The set of valve guides 127 may also function to facilitate alignment of the microfluidic cartridge 100 within an external molecular diagnostic module. In a first embodiment, as shown in FIG. 1B, the set of valve guides 127 comprises holes within the intermediate substrate 120 at the set of occlusion positions 141, with sloped edges configured to direct a pin or indenter through the holes. In the first embodiment, the set of valve guides 127 may be produced in the intermediate substrate 120 by injection molding, or may alternatively be produced by drilling, countersinking, chamfering, and/or beveling. In another embodiment, the set of valve guides 127 comprises

10 and accessible by each fluidic pathway 165 of the micro-

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grooves with holes, such that a pin or indenter is configured to travel along a groove and through a hole that defines the valve guide. In a simplified alternative variation, the set of valve guides 127 may comprise holes through the intermediate substrate 120, wherein the holes do not have sloped edges. In yet another simplified alternative variation, the set of valve guides 127 may comprise a slot configured to provide access to the elastomeric layer 140 by a group of occluding objects (e.g. pins or indenters), rather than a single occluding object.

1.3 Microfluidic Cartridge—Elastomeric and Bottom Layers As shown in FIGS. 1B and 5A-5D, an embodiment of the microfluidic cartridge 100 also comprises an elastomeric layer 140 partially situated on the intermediate substrate  $_{15}$ 120, which functions to provide a deformable substrate that, upon deformation, occludes a microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 at an occlusion position of a set of occlusion positions 141. Preferably, the elastomeric layer 140 comprises an inert, 20 liquid impermeable material, of an appropriate thickness, that can be heated to temperatures encountered during manufacturing and/or specified in a molecular diagnostic protocol, without substantial damage (i.e. compromised surface and/or loss of mechanical robustness) and is chemi- 25 cally compatible with a PCR assay. Preferably, the elastomeric layer 140 is non-continuous, such that portions of the elastomeric layer 140 are positioned relative to the intermediate substrate 120 in a manner that directly covers holes provided by the set of valve guides 127. Alternatively, the 30 elastomeric layer 140 is a continuous layer, spanning a majority of the footprint of the microfluidic cartridge 100 while covering holes provided by the set of valve guides 127. In a specific embodiment, the elastomeric layer 140 comprises 500 micron thick strips of a low-durometer sili- 35 cone that can be heated to at least 120° C. without substantial damage, which are bonded to a portion of the intermediate substrate 120 using a silicone-based adhesive and slightly compressed between the film layer 125 and the intermediate substrate 120. In a variation of the specific 40 embodiment, the elastomeric layer 140 may alternatively be held in place solely by pressure between the intermediate layer 120 and the top layer 110. Preferably, the elastomeric layer 140 is reversibly deformable over the usage lifetime of the microfluidic cartridge 100, such that any occlusion of a 45 microfluidic channel of a fluidic pathway 165 contacting the elastomeric layer 140 is reversible over the usage lifetime of the microfluidic cartridge. Alternatively, the elastomeric layer 140 may not be reversibly deformable, such that an occlusion of a microfluidic channel of a fluidic pathway 165 50 contacting the elastomeric layer 140 is not reversible.

The set of occlusion positions 141 preferably comprises at least two types of occlusion positions, as shown in FIG. 1C, including a normally open position 42 and a normally closed position 43. As shown in FIGS. 5A-5D, the elastomeric layer 55 140 at a normally open position 42 of the set of occlusion positions 141 may be closed upon occlusion by an occluding object (FIGS. 5B and 5D). Preferably, a normally open position 42 is configured to withstand pressures that can be generated by a fluid delivery system (e.g. a syringe pump) 60 without leaking, upon occlusion by an occluding object at the normally open position 42. In one specific example, a ½ barrel-shaped pin head may be used to fully occlude a normally open position 42 having an arched cross section, as in FIG. 5C, with near constant pressure on the portion of the 65 elastomeric layer compressed between the occluding object and occluding position.

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The normally closed position 43 of the set of occlusion positions 141, functions to be normally closed, but to be forced open in response to fluid delivery by a fluid delivery system. In one variation, the normally closed position 43 may be formed by manufacturing (e.g. injection molding) the top layer 100, such that the top layer material at a normally closed position 43 extends down to the elastomeric layer 140. If an occluding object is held away from the normally closed position 43, the occlusion position is closed, but can be forced open due to fluid pressure applied by a fluid delivery system (e.g. syringe pump). When not in operation, however, the normally closed position 43 is configured to prevent leakage and/or fluid bypass. The normally closed position may also be held closed by an occluding object, to prevent leakage even under pressure provided by a fluid delivery system, or under pressure experienced during a high temperature step (e.g., thermocycling) to prevent evaporation of a sample undergoing thermocycling.

The microfluidic cartridge 100 may further comprise a bottom layer 170 configured to couple to the intermediate substrate, which functions to allow waste to be contained within the microfluidic cartridge 100, and allow microfluidic cartridges to be stacked. The bottom layer thus facilitates reception, isolation, and containment of a waste fluid within the waste chamber. Preferably, the bottom layer 170 is composed of the same material as the intermediate substrate 120 for cost and manufacturing considerations, and bonded to the intermediate substrate 120 in a manner that provides a hermetic seal, such that a liquid within the waste chamber 130 does not leak out of the waste chamber 130. In a specific embodiment, the bottom layer 170 and the intermediate substrate 120 are both composed of a polypropylene-based material, and bonded together using an adhesive. In an embodiment of the microfluidic cartridge 100 where the waste chamber 130 has a corrugated surface, the bottom layer 170 preferably only seals voids defining the waste chamber 130, such that non-waste chamber regions (i.e. non-waste housing regions) are not covered by the bottom layer 170. Alternatively, the microfluidic cartridge 100 may omit the bottom layer 170, such that any waste fluid that enters the waste chamber 130 completely leaves the microfluidic cartridge 100 and is collected off-cartridge by a waste-collecting subsystem of an external molecular diagnostic system. In this alternative embodiment, the intermediate substrate 120 is configured to fluidically couple to the waste-collecting subsystem.

#### 1.4 Microfluidic Cartridge—Magnet Housing

The magnet housing region 150 of the microfluidic cartridge 100 functions to provide access to and/or house at least one magnet 152 providing a magnetic field 156 for purification and isolation of nucleic acids. Preferably, the magnet housing region 150 is defined by the film layer and the intermediate substrate, such that the film layer and the intermediate substrate form the boundaries of the magnet housing region 150. In an embodiment of the microfluidic cartridge 100 comprising a bottom layer 170, the magnet housing region 150 may further be defined by the bottom layer 170, such that the bottom layer partially forms a boundary of the magnet housing region 150. The magnet housing region 150 is preferably a rectangular prism-shaped void in the microfluidic cartridge 150, and accessible only through one side of the microfluidic cartridge 100, as shown in FIG. 1B. Preferably, the magnet housing region 150 can be reversibly passed over a magnet 152 to house the magnet 152, and retracted to remove the magnet 152 from the magnet housing region 150; however, the magnet 152 may

alternatively be irreversibly fixed within the magnet housing region 150 once the magnet 152 enters the magnet housing

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Preferably, the magnet housing region 150 is bounded on at least two sides by the waste chamber 130, and positioned 5 near the middle of the microfluidic cartridge 100, such that a fluidic pathway 165 passing through the magnetic field 156 passes through the magnetic field 156 at least at one point along an intermediate portion of the fluidic pathway 165. Preferably, the magnet housing region 150 also substantially 10 spans at least one dimension of the microfluidic cartridge, such that multiple fluidic pathways 165 of the microfluidic cartridge 100 cross the same magnet housing region 150, magnet 152, and/or magnetic field 156. Alternatively, the magnet housing region 150 may be configured such that a 15 magnet within the magnet housing region 150 provides a magnetic field spanning all fluidic pathways 165 of the microfluidic cartridge in their entirety. In alternative embodiments, the microfluidic cartridge may comprise more than one magnet housing region 150, a magnet housing 20 cartridge 100 shown in FIG. 11B, a fluidic pathway 165 is region 150 may be configured to receive and/or house more than one magnet 152, and/or may not be positioned near the middle of the microfluidic cartridge 100. In yet another alternative embodiment, the magnet housing region 150 may permanently house a magnet 152, such that microfluidic 25 cartridge comprises a magnet 152, integrated with the intermediate substrate 120. In embodiments where the magnet 152 is retractable from the microfluidic cartridge 100, the magnet 152 may be a permanent magnet or an electromagnet. In embodiments where the magnet 152 is configured to 30 be integrated with the microfluidic cartridge 100, the magnet 152 is preferably a permanent magnet, which provides a stronger magnetic field per unit volume.

### 1.5 Microfluidic Cartridge—Fluidic Pathways

The set of fluidic pathways 160 of the microfluidic 35 cartridge 100 functions to provide a fluid network into which volumes of sample fluids, reagents, buffers and/or gases used in a molecular diagnostics protocol may be delivered, out of which waste fluids may be eliminated, and by which processed nucleic acid samples may be delivered to a 40 detection chamber for analysis, which may include amplification and/or detection. Preferably, each fluidic pathway 165 in the set of fluidic pathways 160 is formed by at least a portion of the top layer, a portion of the film layer, and a portion of the elastomeric layer 140, such that each fluidic 45 pathway 165 may be occluded upon deformation of the elastomeric layer 140 at a set of occlusion positions 141. Additionally, at least one fluidic pathway 165 in the set of fluidic pathways 160 is preferably fluidically coupled to a sample port-reagent port pair 113 of the set of sample 50 port-reagent port pairs 112, a fluid port 118, a waste chamber 130, and a detection chamber 117 of the set of detection chambers 116. Furthermore, at least one fluidic pathway 165 in the set of fluidic pathways 160 is preferably configured to be occluded upon deformation of the elastomeric layer 140, 55 configured to transfer a waste fluid to the waste chamber 30, comprises a capture segment 166 passing through the heating region 195 and a magnetic field 156, and is configured to pass through the vent region 190 upstream of a detection chamber 117. Alternative embodiments may omit preferred 60 elements of the embodiment of the fluidic pathway 165 described above, such as a vent region 190 or a heating region 195, or add additional elements to the embodiment of the fluidic pathway 165 described above.

A fluidic pathway 165 of the set of fluidic pathways 160 65 may comprise portions (i.e. microfluidic channels) that are located on both sides of the top layer 110, but is preferably

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located primarily on the bottom side of the top layer (in the orientation shown in FIG. 1B). In the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a microfluidic channel on top of the top layer 110 may be further covered by second film layer 168 that seals the microfluidic channel on top of the top layer 110. The second film layer 168 may be comprise a cyclic olefin polymer (COP) film, thermally or adhesively bonded to the top layer 110, or alternatively may comprise another material that is bonded to the top layer 110. The use of film layers 125, 168 to cover microfluidic channels on either side of the top layer 110 facilitates manufacturing, such that long stretches of a fluidic pathway 165 do not need to be produced within the interior of the top layer 110. Preferably, microfluidic channels may be etched, formed, molded, cut, or otherwise shaped into the rigid structure of the top layer 110, and either remain on one side of the top layer 110, or pass through the thickness of the top layer 110.

In one variation, in the orientation of the microfluidic preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a vent region 190 on the top side of the top layer 110. All other segments of the fluidic pathway 165 are preferably located on the bottom side of the top layer 110, allowing the fluidic pathway 165 to be sealed by the film layer 125 without requiring a separate film layer to seal channels located on the top of the top layer 110.

In another variation, in the orientation of the microfluidic cartridge 100 shown in FIG. 1B, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising a segment running to a detection chamber 163 on the top side of the top layer 110 and a segment running away from the detection chamber 164 on the top side of the top layer 110. In this variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 upstream of the first segment running to the detection chamber 163, and crosses the thickness of the top layer 110 downstream of the segment running away from the detection chamber 164, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. In another variation, as shown in FIG. 6C, a fluidic pathway 165 is preferably located primarily on the bottom side of the top layer 110, comprising only a segment running away from the detection chamber 164 on the top side of the top layer 110. In this other variation, the fluidic pathway 165 thus crosses the thickness of the top layer 110 downstream of the second portion, and crosses the thickness of the top layer 110 to couple to a sample port 114 and a reagent port 115 on the top side of the top layer 110. Alternatively, other embodiments may comprise a fluidic pathway 165 with a different configuration of portions on the top side of the top layer 110 and/or portions on the bottom side of the top layer 110.

As shown in FIGS. 1C, 6C, 7 and 9, a fluidic pathway 165 of the set of fluidic pathways 160 is branched and preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118, a sample segment 175 coupled to a sample port 114, a reagent segment 176 coupled to a reagent port 115, a capture segment 166 passing through at least one of the heating region 195 and a magnetic field 156, a vent segment 177 configured to pass through the vent region 190, a segment running to a detection chamber 163, a segment running away from the detection chamber 164, and at least one waste segment 178, 179 configured to transfer a waste fluid to a waste chamber 130. Individual segments of the fluidic

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pathway **165** are preferably configured to pass through at least one occlusion position of the set of occlusion positions **141**, to controllably direct fluid flow through portions of the fluidic pathway **165**. A fluidic pathway **165** may also further comprise an end vent **199**, which functions to prevent any fluid from escaping the microfluidic channel.

The initial segment 174 of the fluidic pathway 165 functions to deliver common liquids and/or gases from a fluid port 118 through at least a portion of the fluidic pathway 165, the sample segment 175 functions to deliver a volume of a sample fluid (e.g. sample comprising nucleic acids bound to magnetic beads) to a portion of the fluidic pathway 165, and the reagent segment 176 functions to deliver a volume of fluid comprising a reagent to a portion of the fluidic pathway 165. The capture segment 166 functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid, and may be s-shaped and/or progressively narrowing, to increase the efficiency and/or effectiveness of isolation and purification. Alterna- 20 tively, the capture segment 166 may altogether be replaced by a substantially straight portion 166 or any other geometric shape or configuration that functions to facilitate isolation and purification of nucleic acids from the volume of the sample fluid. The capture segment 166 of the fluidic path- 25 way 165 preferably has an aspect ratio less than one, which functions to facilitate capture of magnetic particles, but may alternatively have an aspect ratio that is not less than one.

The vent segment 177 functions to deliver a processed sample fluid through the vent region 190 for gas removal. 30 The segment running to a detection chamber 163 functions to deliver a processed sample fluid to the detection chamber 117 with a reduced quantity of gas bubbles, and the segment running away from the detection chamber 164 functions to deliver a fluid away from the detection chamber 117. The 35 segments may be arranged in at least one of several configurations to facilitate isolation, processing, and amplification of a nucleic acid sample, as described in three exemplary embodiments below:

A first embodiment, as shown in FIG. 1C, of a fluidic 40 pathway 165 preferably comprises an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a shared fluid port 118, a sample segment 175 coupled to a sample port 114 and to the initial segment 174, and an s-shaped capture segment 166, configured to pass through the heating 45 region 195 and a magnetic field 156, coupled to the initial segment 174 and the sample segment 175. In a variation of the first embodiment, the s-shaped capture segment 166 may comprise an initial wide arc 166 to provide a greater surface area for magnetic bead capture. In another variation of the 50 first embodiment, the capture segment 166 may alternatively be a progressively narrowing s-shaped capture segment 166. The first embodiment of the fluidic pathway 165 also comprises a reagent segment 176 coupled to a reagent port 115 and to the capture segment 166, a vent segment 177 55 coupled to the reagent segment 176 and configured to pass through the vent region 190, a segment running to a detection chamber 163 from the vent region 190, a winding segment running away from the detection chamber 164, and an end vent 199 coupled to the segment running away from 60 the detection chamber 164. The first embodiment of the fluidic pathway 165 also comprises a first waste segment 178 configured to couple the initial segment 174 to the waste chamber 130, and a second waste segment 179 configured to couple the capture segment 166 to the waste chamber 130. 65 The first waste segment 178 preferably functions to allow evacuation of excess release fluids from a fluidic pathway

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165, for precise metering of the amount of release reagents used in a molecular diagnostic procedure using a low volume of sample.

In the first embodiment, the set of occlusion positions 141 comprises a first occlusion position 142 located along the initial segment 174 between points at which the initial segment couples to the fluid channel 119 and to the capture segment 166. The set of occlusion positions 141 also comprises a second occlusion position 143 located along the sample segment 175, a third occlusion position 144 located along the reagent segment 176, a fourth occlusion position 145 located along the first waste segment 178, and a fifth occlusion position 146 located along the second waste segment 179. In the first embodiment, the set of occlusion positions 141 also comprises a sixth occlusion position 147 located along the vent segment 177 upstream of the vent region 190, a seventh occlusion position 148 located along the segment running to the detection chamber 163, and an eighth occlusion position 149 located along the segment running away from the detection chamber 164. In the first embodiment, the first, second, third, fifth, and sixth occlusion positions 142, 143, 144, 146, 147 are normally open positions 42 and the fourth, seventh, and eighth occlusions positions 145, 148, 149 are normally closed positions 43, as shown in FIG. 1C.

The occlusion positions of the set of occlusion positions 141 of the first embodiment are preferably located such that occluding subsets of the set of occlusion positions 141 defines unique truncated fluidic pathways to controllably direct fluid flow. For example, as shown in FIG. 1D, occluding the fluidic pathway 165 at the first, third, fourth, and sixth occlusion positions 142, 144, 145, 147 forms a truncated pathway by which a volume of a sample fluid, comprising nucleic acids bound to magnetic beads and delivered into the sample port 114, may flow past the second occlusion positions 143 into the capture segment 166 for isolation and purification of nucleic acids using the heating region 195 and the magnetic field 156. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment 166 by the magnetic field 156, while other substances in the volume of sample fluid may pass into the waste chamber 130 by passing the fifth occlusion position **146**. Following this subset of occlusion positions, the occlusion at the first occlusion position 142 may be reversed, as shown in FIG. 1E, and the fluidic pathway 165 may be occluded at the second occlusion position 143 to form a second truncated pathway by which a wash fluid may be delivered through the fluid port 118, into the capture segment 166 (thus washing the trapped magnetic beads), and into the waste chamber 130 by passing the fifth occlusion position 146. The occlusion at the second occlusion position 143 may then be reversed, and the first occlusion position 142 may be occluded (as shown in FIG. 1D), so that other fluidic pathways in the set of fluidic pathways 160 may be washed. After all fluidic pathways have been washed, a volume of air may be transferred through the fluid port 118 to prevent mixture of a wash solution with a release solution.

Thereafter in the first embodiment, as shown in FIG. 1E, the fluidic pathway 165 may be occluded at the second occlusion position 143 and the occlusion at the first occlusion 142 may be reversed, thus creating a third truncated pathway as shown in FIG. 1D. A release solution may then be delivered through the fluid port 118, into the capture segment 166, and to the waste chamber 130 by passing the fifth occlusion position 146. The release solution may then be sealed within a fourth truncated pathway (including the capture segment 166) of the fluidic pathway 165 by occlud-

ing the fluidic pathway at the fifth occlusion position 146, as shown in FIG. 1F. A release solution may then be delivered to other fluidic pathways of the set of fluidic pathways 160.

Thereafter, as shown in FIG. 1G, the occlusion at the fourth occlusion position 145 may be reversed, creating a 5 fifth truncated pathway, and release solution within the fluidic pathway 165 may be metered by pumping air through the fluid port 118, which functions to push a portion of the release solution into the waste chamber 130. A volume of release solution will still be maintained within the capture 10 segment 166 at this stage. As shown in FIG. 1H, the first and the fourth occlusion positions 142, 145 may then be occluded to form a sixth truncated pathway sealing the volume of release solution, with the captured magnetic beads bound to nucleic acids, within the capture segment 15 166. The volume of the remaining release solution is therefore substantially defined by the microchannel volume between junctions in the fluidic pathway 165 near the fourth and sixth occlusion positions 145, 147, and may be any small volume but in a specific variation is precisely metered 20 to be 23+/-1 microliters. Release solution may be sealed within capture segments of other fluidic pathways using a similar process. A heater may then be provided at the sixth truncated pathway, inducing a pH shift within the sixth truncated pathway to unbind nucleic acids from the mag- 25 netic beads.

Thereafter in the first embodiment, as shown in FIG. 1I, the occlusions at the first and third occlusion positions 142, 144 may be reversed, defining a seventh truncated pathway, and the entire released nucleic acid sample (e.g. ~20 micro- 30 liters) may be aspirated out of the microfluidic cartridge through the reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent stored off of the microfluidic cartridge 100. During the reconstitution, the occlusion at the sixth occlusion posi- 35 tion 147 may be reversed, and the fluidic pathway 165 may be occluded at the first occlusion position 142 to form an eighth truncated pathway, as shown in FIG. 1J. Once reconstitution of the molecular diagnostic reagent with the released nucleic acid sample is complete and well mixed, the 40 reconstituted mixture may then be dispensed through the reagent port 115, through the eighth truncated pathway, and to the detection chamber 117, by using a fluid handling system to push the seventh occlusion position (normally closed) open. The detection chamber 117 is completely filled 45 with the mixed reagent-nucleic acid sample, after which the fluidic pathway 165 is occluded at the third, sixth, seventh and eighth occlusion positions 144, 147, 148, 149, defining ninth truncated pathway, as shown in FIG. 1K. Other pathways of the set of fluidic pathways 165 may be similarly 50 configured to receive a reagent-nucleic acid mixture. An external molecular diagnostic system and/or module may then perform additional processes, such as thermocycling and detection, on the volume of fluid within the detection

An alternative variation of the first embodiment may further comprise additional occlusion positions or alternative variations of the set of occlusion positions 141, such that occlusion at the additional occlusion positions permanently seals the waste chamber from the fluidic pathway 165. Other 60 alternative variations of the first embodiment may also comprise configurations of the set of occlusion positions 141 that are different than that described above. The variations may be configured, such that the a fluidic pathway 165 facilitates meter release, does not allow meter release, 65 facilitates addition of other reagents (e.g. neutralization or DNase reagents), facilitates additional washing steps, and/or

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facilitates other operations without changing the layout of the fluidic pathway 165 of a microfluidic cartridge embodiment. Thus, multiple unique operations may be performed using the same microfluidic cartridge, by occluding fluidic pathways 160 at varied subsets of a set of occlusion positions 141.

A second embodiment, as shown in FIG. 6C, of a fluidic pathway 165' preferably comprises an initial segment 174' fluidically coupled to a fluid channel 119' coupled to a shared fluid port 118', a sample segment 175' coupled to a sample port 114' and to the initial segment 174', and a capture segment 166', configured to pass through the heating region 195 and a magnetic field 156, coupled to the initial segment 174'. The second embodiment of the fluidic pathway 165' also comprises a reagent segment 176' coupled to a reagent port 115' and to the turnabout portion 176', a vent segment 177' coupled to the reagent segment 176' and to the capture segment 166' and configured to pass through the vent region 190, a segment running to a detection chamber 163' from the vent region 190, a segment running away from the detection chamber 164', and an end vent 199 coupled to the segment running away from the detection chamber 164'. The second embodiment of the fluidic pathway 165' also comprises a first waste segment 178', coupled to the initial segment 174' at a point between points connecting the initial segment 174' to the sample segment 175' and to the capture segment 166'. The first waste segment 178' is configured to couple the initial segment 174' to the waste chamber 130. The second embodiment of the fluidic pathway 165' also comprises a second waste segment 179' configured to couple the capture segment 166' to the waste chamber 130', and an end vent segment 197' coupled to the capture segment 166' downstream of the point of connection to the second waste segment 179', and coupled to an end vent 199. The end vent segment 197' functions to provide fine metering of a fluid flowing through the fluidic pathway 165'.

In the second embodiment, the set of occlusion positions 141' comprises a first occlusion position 142' located along the initial segment 174' between points at which the initial segment couples to the fluid channel 119' and to the sample segment 175'. The set of occlusion positions 141' also comprises a second occlusion position 143' located along the sample segment 175', a third occlusion position 144' located along the reagent segment 176', a fourth occlusion position 145' located along the first waste segment 178', and a fifth occlusion position 146' located along the second waste segment 179'. In the second embodiment, the set of occlusion positions 141' also comprises a sixth occlusion position 147' located along the vent segment 177' upstream of the vent region 190, a seventh occlusion position 148' located along the segment running to the detection chamber 163', and an eighth occlusion position 149' located along the segment running away from the detection chamber 164'. Additionally, in the second embodiment, the set of occlusion positions 141 comprises a ninth occlusion position 157' located along the sample segment 175' between the sample port 114 and the second occlusion position 143, a tenth occlusion position 158' located along the end vent segment 197', and an eleventh occlusion position 159' located along the capture segment 166' between points at which the capture segment 166' couples to the end vent segment 197' and to the vent segment 177'.

The occlusion positions of the set of occlusion positions 141' of the second embodiment are preferably located such that occluding of subsets of the set of occlusion positions 141' defines unique truncated fluidic pathways to controllably direct fluid flow. For example, occluding the fluidic

pathway 165' at the first, fourth, sixth, tenth, and eleventh occlusion positions 142', 145', 147', 158', 159' forms a truncated pathway by which a volume of a sample fluid,

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comprising nucleic acids bound to magnetic beads and delivered into the sample port 114, may flow into the capture 5 segment 166' for isolation and purification of nucleic acids using the heating region 195 and the magnetic field 156. Nucleic acids bound to magnetic beads may thus be trapped within the capture segment 166' by the magnetic field 156, while other substances in the volume of sample fluid may 10 pass into the waste chamber 130 by passing the fifth occlusion position 146'. Following this subset of occlusion positions, the occlusion at the first occlusion position 142' may be reversed, and the fluidic pathway 165' may be occluded at the second occlusion position 143' to form a second 15 truncated pathway by which a wash fluid may be delivered through the fluid port 118, into the capture segment 166' (thus washing the trapped magnetic beads), and into the waste chamber 130 by passing the fifth occlusion position **146'**. A volume of air may then be pumped through the fluid 20 port 118 to flush any remaining wash solution into the waste chamber 130.

Thereafter, in the second embodiment, the fluidic pathway 165' may be occluded at the fifth occlusion position 146' and the occlusion at the tenth occlusion position 158' may be 25 reversed, closing access to the waste chamber 130 and opening access to the end vent segment 197'. A release solution may then be delivered through the fluid port 118, into the capture segment 166', and to the end vent segment **197**'. The volume of the release solution is therefore defined 30 by the microchannel volume between the fourth and tenth occlusion positions 145', 158', and may be any small volume but in a specific variation is precisely metered to be 15 microliters. Thereafter, occluding the fluidic pathway 165' at the tenth occlusion position 158', reversing the occlusion at 35 the fourth occlusion position 145' (defining a fourth truncated pathway), and delivering air through the fluid port 118 pushes any remaining release buffer from the fluidic pathway 118 into the waste chamber 130, thereby ensuring that excess release buffer is not later exposed to nucleic acids 40 bound to the magnetic beads (at this point, the nucleic acids are not substantially released from the magnetic beads because heat has not been added). Thereafter, the fluidic pathway 165' is occluded at the first and fourth occlusion positions 142', 145', defining a fifth truncated pathway 45 comprising the capture segment 166', and the magnetic beads are heated to an appropriate temperature and time (e.g., 60 degrees for 5 minutes) within the heating region 195 to release the nucleic acids from the magnetic beads and into the release buffer.

Thereafter, in the second embodiment, the occlusions at the first and eleventh occlusion positions 142', 159' are reversed, defining a sixth truncated pathway, the entire released nucleic acid sample (e.g. ~15 microliters) may be aspirated out of the microfluidic cartridge through the 55 reagent port 115. This released nucleic acid sample is then used to reconstitute a molecular diagnostic reagent mixture stored off of the microfluidic cartridge 100. During the reconstitution process, the occlusion at the sixth occlusion position 147' may be reversed, thus defining a seventh 60 truncated pathway. Once reconstitution of the molecular diagnostic reagent mixture with the released nucleic acid sample is complete and well mixed, the reconstituted mixture may then be aspirated through the reagent port 115 through the seventh truncated pathway to the detection 65 chamber 117, completely filling the detection chamber 117, after which the fluidic pathway 165' is be occluded at third,

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seventh, eighth, and ninth occlusion positions 144', 148', 149', 157' defining an eighth truncated pathway. An external molecular diagnostic system and/or module may then perform additional processes on the volume of fluid within the detection chamber 117.

An alternative variation of the second embodiment may further comprise additional occlusion positions or alternative variations of the set of occlusion positions 141', such that occlusion at the additional occlusion positions permanently seals the waste chamber from the fluidic pathway 165'. Other alternative variations of the second embodiment may also comprise configurations of the set of occlusion positions 141' that are different than that described above.

A third embodiment, as shown in FIG. 7, of a fluidic pathway 165" preferably comprises an initial segment 174" fluidically coupled to a fluid channel 119" coupled to a shared fluid port 118, a sample segment 175" coupled to a sample port 114 and to the initial segment 174", and a capture segment 166" coupled to the initial segment 174". The third embodiment of the fluidic pathway 165" also comprises a reagent segment 176" coupled to a reagent port 115, a vent segment 177" coupled to the reagent segment 176" and to the capture segment 166", and configured to pass through the vent region 190, a segment running to a detection chamber 163" from the vent region 190, a segment running away from the detection chamber 164", and an end vent 199 coupled to the segment running away from the detection chamber 164". The third embodiment of the fluidic pathway 165" also comprises a first waste segment 178" configured to couple the initial segment 174" to the waste chamber 130, and a second waste segment 179" configured to couple the capture segment 166" to the waste chamber 130.

In the third embodiment, the set of occlusion positions 141" comprises a first occlusion position 142" located along the initial segment 174" between points at which the initial segment 174" couples to the fluid channel 119" and to the sample segment 175". The set of occlusion positions 141" also comprises a second occlusion position 143" located along the sample segment 175", a third occlusion position 144" located along the reagent segment 176", a fourth occlusion position 145" located along the first waste segment 178", and a fifth occlusion position 146" located along the second waste segment 179". In the third embodiment, the set of occlusion positions 141" also comprises a sixth occlusion position 147" located along the vent segment 177" upstream of the vent region 190, a seventh occlusion position 148" located along the segment running to the detection chamber 163", an eighth occlusion position 149" located along the segment running away from the detection chamber 164", and a ninth occlusion position 157" located along the vent segment 177" between the point at which the vent segment 177" couples to the second waste segment 179" and the sixth occlusion point 147".

Similar to the first and the second embodiments, the occlusion positions of the set of occlusion positions 141" of the third embodiment are preferably located such that an occlusion of subsets of the set of occlusion positions 141" defines unique truncated fluidic pathways to controllably direct fluid flow. Example truncated fluidic pathways, defined by occluding the fluidic pathway 165" using subsets of the set of occlusion positions 141", are shown in FIG. 7.

Preferably, a fluidic pathway 165 of the set of fluidic pathways 160 comprises at least one of a first channel type 171, a second channel type 172 with a reduced cross sectional area, and a third channel type 173 with an curved surface as shown in FIG. 8A. A variation of the first channel

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type 171 has an approximately rectangular cross section with slightly sloping walls, such that at least two walls of the first channel type 171 slope toward each other to facilitate manufacturing of the first channel type 171; however, alternative variations of the first channel type 171 may have 5 non-sloping walls or walls that slope away from each other. In specific embodiments of the first channel type 171, the walls of the first channel type 171 slope at 6° from vertical, to facilitate extraction of injection molded parts, and are between 300 and 1600 microns wide and between 100 and 475 microns tall. In a first specific embodiment of the second channel type 172, the cross section of the second channel type 172 is a 250 micron wide equilateral triangle with the top truncated to be 200 microns deep. In a second specific embodiment of the second channel type 172, the cross section of the second channel type is a truncated triangle that is 160 microns wide and 160 microns deep. In a specific embodiment of the third channel type 173, the surface of the third channel type is defined by Gaussian function, and is 800 microns wide and 320 microns deep. Alternative 20 embodiments of the third channel type 173 may comprise a surface defined by any appropriate curved function.

The first channel type 171 is preferably used over a majority of a fluidic pathway 165, and preferably in portions near a vent region 190, in a capture segment 166 configured 25 to pass through a magnetic field 156, and in a segment leading to a Detection chamber 163. Preferably, an embodiment of the first channel type 171, comprising a wide channel with little depth is used in regions configured to pass through a magnetic field 156, such that particles in the 30 regions are driven closer to the magnetic field source. The second channel type 172 is preferably used near a vent region 190 of a fluidic pathway 165, and preferably in portions of a fluidic pathway 165 leading to and away from a detection chamber 163, 164 (to constrict fluid flow into the 35 Detection chamber 117). The third channel type 173 is preferably used in a portion of a fluidic pathway 165 near a normally open position 42 of the set of occlusion positions 141. Transitions between different channel types 171, 172, 173 may be abrupt, or alternatively, may be gradual, as 40 shown in FIG. 8B. The first, second, and third channel types 171, 172, 173 may also alternatively be used in any appropriate portion of a fluidic pathway 165. Example embodiments of channel types for segments of a fluidic pathway are shown in FIG. 8C.

Multiple fluidic pathways may be configured to pass through a single heating region 195 of the microfluidic cartridge 100, a single vent region 190 of the microfluidic cartridge 100, and/or a magnetic field 156 produced by a magnet 152 housed within a single magnet housing region 50 150. Preferably all fluidic pathways of the set of fluidic pathways 160 are configured to pass through a single heating region 195 of the microfluidic cartridge 100, a single vent region 190 of the microfluidic cartridge 100, and a magnetic field 156 produced by a magnet 152 housed within a single 55 magnet housing region 150; however, alternative embodiments of the set of fluidic pathways 160 of the microfluidic cartridge may comprise different configurations wherein fluidic pathways of the set of fluidic pathways 160 do not share a single heating region 195, a single vent region 190, 60 and/or a magnetic field 156.

Additionally, the set of fluidic pathways 160 of the microfluidic cartridge 100 may comprise virtually any number of fluidic pathway 165 and/or the set of Detection chambers 116 may comprise virtually any number of Detection chambers 116 as can practically be integrated into the microfluidic cartridge 100. In one specific embodiment, the

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set of fluidic pathways **160** may comprise twelve fluidic pathways **165**, four of which are shown in FIG. **9**. 1.6 Microfluidic Cartridge—Additional Microfluidic Car-

The microfluidic cartridge 100 is preferably configured such that actual valving members are not integrated into the microfluidic cartridge 100, thus, opening and/or occluding portions of a fluidic pathway 165 are performed by systems located external to the microfluidic cartridge. As an example, portions of a fluidic pathway 165 may be opened or occluded at occlusion positions, as described above, by the action of a valving member or mechanism held beneath the card that applies a biasing force to deform the elastomeric layer 140 and occlude a fluidic pathway 165. The force may be applied by a mechanical member (e.g., a pin, post, etc.), an electromechanical member (e.g. a solenoid), a pneumatic or hydraulic member (e.g., air, water, etc.) or any other appropriate means, as shown in FIGS. 10A and 10B. In some variations, the cartridge may include one or more registration regions that allow the card to be aligned with respect to the valving member or mechanism. In alternative embodiments, the elastomeric layer 140, the set of valve guides 127, and the set of occlusion positions 141 may be omitted and replaced with valves integrated within the microfluidic cartridge 100, that are configured to controllably occlude and open portions of a fluidic pathway 165.

Other embodiments of the microfluidic cartridge 100 may further comprise a tag 198 that functions to encode and provide identifying information related to the microfluidic cartridge 100. The tag 198 may comprise a barcode, QR code, or other optical machine-readable tag, or may alternatively be an electronic tag, such as an RFID chip. The identifying information preferably comprises at least information relating to the position of a microfluidic cartridge 100 within a molecular diagnostic system, and information relating to samples analyzed using the microfluidic cartridge 100 (e.g. how many positions remain available for conducting tests). In alternative variations, the tag may relate other information about samples (e.g. sample type, sample volume, sample concentration, date) processed using the microfluidic cartridge 100. Preferably, the tag does not interfere with procedures being performed using the microfluidic cartridge, and is located in an unobtrusive position on the microfluidic cartridge 100, such as a side panel of the microfluidic cartridge 100. Alternatively, the microfluidic cartridge 100 may not comprise a tag 198, and a user or other entity may relate identifying information to the microfluidic cartridge 100 using any appropriate element.

As a person skilled in the art will recognize from the previous detailed description and from the FIGURES and claims, modifications and changes can be made to the preferred embodiments of the microfluidic cartridge 100 without departing from the scope of this invention, as is shown in the example embodiment shown in FIGS. 11A and 11B, and in the alternative example embodiment of FIGS. 6A-6C, wherein in the orientation of FIG. 6B, the intermediate substrate 120 comprising a waste chamber 130 is coupled to the top layer 110, and the elastomeric layer 140 is located on the bottom of the microfluidic cartridge 100. 2. Specific Embodiment of a Microfluidic Cartridge

The following description a specific embodiment of the microfluidic cartridge 100 is for illustrative purposes only, and should not be construed as definitive or limiting of the scope of the claimed invention.

The specific embodiment of the microfluidic cartridge **100**, as shown in FIGS. **11**A and **11**B, meets SLAS ANSI guidelines for a microtiter plate footprint, governing the

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dimensions of the specific embodiment of the microfluidic cartridge 100. The specific embodiment of the microfluidic cartridge 100 is thus 127.76 mm long and 85.48 mm wide.

The specific embodiment of the microfluidic cartridge 100 comprises a top layer 110 including a set of twelve sample port-reagent port pairs 112, a set of twelve Detection chambers 116, a shared fluid port 118, a heating region 195, and a vent region 190, an intermediate substrate 120, coupled to the top layer 110 and partially separated from the top layer 110 by a film layer 125, configured to form a waste chamber 130, an elastomeric layer 140 partially situated on the intermediate substrate 120; a magnet housing region 150 accessible by a magnet 152 providing a magnetic field 156; a bottom layer 170 coupled to the intermediate substrate 120 and configured to seal the waste chamber, and a set of fluidic pathways 160, formed by at least a portion of the top layer 110, a portion of the film layer 125, and a portion of the elastomeric layer 140.

The top layer 110 of the specific embodiment of the 20 microfluidic cartridge 100 functions preferably as described in Section 1.1, and is composed of polypropylene with low autofluorescence and a glass transition temperature suitable for PCR. The majority of the top layer 110 of the specific embodiment is 1.5 mm thick (aside from regions defining 25 ports, the vent, the heating region 195 or fluidic pathways 165), and is produced by injection molding without the use of a mold release. The polypropylene is clear to allow transmission of light in the detection chambers. The injection molding process defines the set of 12 sample port- 30 reagent port pairs, which are located along one long edge of the top layer 110, and also defines the set of 12 detection chambers 116, which are located along the opposite long edge of the top layer 110. The Detection chambers 117 do not completely transect the top layer 110, as shown in FIGS. 35 11A and 11B. Each detection chamber 117 of the specific embodiment is identical and comprised of three interconnected channels, configured in a circular arrangement, with each of the interconnected channels approximately 0.4 mm deep and 1.6 mm wide at its widest point, resulting in a total 40 volume of ~10 mL for each detection chamber 117. The dimensions of the detection chambers 117 of the specific embodiment are such that the detection chambers 117 facilitate heating from one side (resulting in simpler heater design yet fast cycling given the small depth of the channels), and 45 also facilitate the injection molding process. The bottoms of the detection chambers 117 are formed by the film layer 125. which is polypropylene film compatible with PCR (100 microns thick or less) that offers low autofluorescence. The film layer 125 can withstand temperatures up to 120° C. or 50

The injection molding process also defines the shared fluid port 118 of the top layer 110, and the vent region 190, which is recessed 0.5 mm into the top surface of the top layer 110 (in the orientation shown in FIG. 11B), and is covered 55 with a polytetrafluoroethylene membrane, which is hydrophobic, gas permeable, and liquid impermeable. A paper label is bonded with adhesive to the top layer 110 over the vent region 190, which serves to identify the cartridge and protect the vent region 190, as shown in FIGS. 11A and 11B. 60 The injection molding process also defines the heating region 195, which is recessed and spans the long dimension of the top layer 110, slightly offset from a midline of the top layer 110. The top layer 110 of the specific embodiment requires approximately 15 grams of polypropylene, and all 65 draft angles for the top layer 110 are a minimum of 4 degrees, as defined by the injection molding process.

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In the specific embodiment, the intermediate substrate 120 is composed of a polypropylene material to minimize cost and simplify assembly, and in the orientation shown in FIG. 11B, the top of the intermediate substrate 120 is 1.5 mm thick. The film layer 125, partially separating the intermediate substrate 120 from the top layer 110 is a polypropylene film with a nominal thickness of 50 microns. The film layer 125 is able to withstand temperatures of up to 95° C. encountered during fabrication and during an intended PCR procedure, while being thermally bondable to the top layer 110. The top layer 110 and the film layer 125 are bonded using thermal fusion bonding, and this subassembly is bonded to the intermediate substrate 120 using a polymer adhesive. Additionally, for aligning layers 110, 120, 125 and bonding the top layer 110 to the intermediate substrate 120, plastic studs are configured to extend from the top of the intermediate substrate 120 through die-cut holes in the film layer 125 and injection molded holes in the bottom of the top layer 110. The intermediate substrate also comprises a set of valve guides 127, at a set of occlusion positions 141, which are holes with chamfered edges through the intermediate substrate 127. Each valve guide in the set of valve guides 127 is 2.1 mm×2.1 mm square, and configured to accommodate an occluder with a 2 mm×2 mm square head for normally open positions 42 or 2.1 mm diameter circle to accommodate a 2 mm diameter round pin for normally closed positions 43.

The elastomeric layer 140 of the specific embodiment is composed of a low durometer silicone, and comprises strips that are 500 microns thick and that can withstand temperatures of 120° C. at a minimum. The strips of the elastomeric layer are arranged over the set of valve guides 127, and bonded to the top of the intermediate substrate 120 using a silicone adhesive. Additionally, the elastomeric layer 140 is slightly compressed between the film layer 125 and the top of the intermediate substrate (in the orientation shown in FIG. 11B).

The bottom layer 170 of the specific embodiment of the microfluidic cartridge 100 is composed of polypropylene, identical to that of the intermediate substrate 120. The bottom layer is 1.5 mm thick, and is contiguous in the area of the set of Detection chambers 116, such that an outer perimeter of the entire bottom layer 170 substantially spans the footprint of the microfluidic cartridge 100. The bottom layer 170 of the specific embodiment is bonded to the intermediate substrate 120 using polymer adhesive, providing a hermetic seal that ensures that a waste fluid within the waste chamber 130 of the intermediate substrate 120 does not leak out of the waste chamber 130.

The specific embodiment of the microfluidic cartridge 100 comprises twelve fluidic pathways 165 in the set of fluidic pathways 160, such that the microfluidic cartridge 100 is capable of testing up to twelve samples using twelve distinct fluidic pathways **165**. Each of the twelve fluidic pathways 165 is coupled to one of the twelve sample port-reagent port pairs 113 on one end of the microfluidic cartridge 100, and coupled to one of the twelve detection chambers 117 on the other end of the microfluidic cartridge, as shown in FIGS. 11A and 11B. Each fluidic pathway 165 is substantially identical (aside from portions connecting to an initial segment 174 fluidically coupled to a fluid channel 119 coupled to a fluid port 118) and identical to the first embodiment of a fluidic pathway described in Section 1.5 and shown in FIG. 1C. Additionally, the microfluidic channels comprising each fluidic pathway 165 are of the first channel type 171 and 500 microns wide by 475 microns deep, aside from the microfluidic channels of the segments leading to and away from 25

the detection chambers 163, 164, the turnabout portions 166, and the vent segments 177. Also, parallel microfluidic channels of the fluidic pathways 165 of the specific embodiment are typically evenly spaced at 2.25 mm (center-to-center).

The fluidic pathways 165 of the specific embodiment are, in their default condition, open at all occlusion positions, aside from the fourth, seventh, and eighth, occlusion positions 145, 148, 149, as shown in FIG. 1C. Furthermore, the s-shaped capture segment 166 of a fluidic pathway of the 10 specific embodiment is configured to have a volume capacity of 22  $\mu$ L, have a width of 5.5 mm, and weave back and forth over a magnetic field 156, by crossing the magnet housing region 150. The depth of the s-shaped capture segment 166 is 0.4 mm for the 1.6 mm wide channels and 15 0.475 for the 0.5 mm narrower channel.

The specific embodiment also comprises a barcode tag 198 located on a vertical edge of the microfluidic cartridge 100, as shown in FIG. 11A. Additional features of the specific embodiment of the microfluidic cartridge 100 are 20 shown in FIGS. 11A and 11B.

3. Assembly Method for an Embodiment of the Microfluidic
An embodiment of an assembly method 200 for an embodiment of the microfluidic cartridge 100 is shown in FIGS. 12A-12G. The assembly method 200 preferably comprises aligning the top layer to the film layer and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge S210; compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate and bonding the top/film layers to the elastomeric layer/intermediate substrate S220, bonding the intermediate substrate to the bottom layer S230; installing the vents of the vent region S250; and applying labels and packaging S260.

Step S210 recites aligning the top layer to the film layer 35 and thermally bonding the two, using silicone adhesive to bond the elastomeric layer to the intermediate substrate of the microfluidic cartridge, and functions to create a first subassembly comprising the top layer, the film layer, the elastomeric layer, and the intermediate substrate. Preferably, 40 the elastomeric layer is glued with silicone to the intermediate substrate; however, the elastomeric layer may alternatively be solely compressed between the top layer/film layer and the intermediate substrate, without any adhesive. Preferably, a first jig is used to align the top layer and the film 45 layer using pins in the jig and holes in the layers, and in an example embodiment of S210, the top layer is first placed face down in the first jig, and the film layer is placed onto the top layer in preparation for thermal bonding using a lamination machine or hot press. In the example embodi- 50 ment of S210, the elastomeric layer is then fit over ultrasonic welding tabs in of the top layer, as shown in FIGS. 12D and 12F, however, processes other than ultrasonic welding may be used. An adhesive may also be applied around the border of the elastomeric layer, to prevent leakage between the 55 elastomeric layer and the intermediate substrate. Protrusions molded into the top of the intermediate substrate are then passed through alignment holes in the top layer, thus aligning the top layer, the elastomeric layer, and the intermediate substrate of the microfluidic cartridge. In alternative 60 embodiments of S210, any appropriate alignment mechanism may be used to align the top layer, the elastomeric layer, and the intermediate substrate, using for example, a combination of adhesives, frames, and alignment pins/re-

Step S220 recites compressing the top layer, the film layer, the elastomeric layer, and the intermediate substrate

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and bonding the top/film layers to the elastomeric layer/intermediate substrate, and functions to seal the layers in order to prevent leakage between the layers. Preferably, S220 forms hermetic seals between the top layer and the elastomeric layer, and the elastomeric layer and the intermediate substrate, in embodiments of S210 where an adhesive application is involved. In an example embodiment of S220, the first jig with the top layer, the elastomeric layer, and the intermediate substrate is placed within an ultrasonic welder to be compressed and ultrasonically welded.

Step S230 recites bonding the intermediate substrate to the bottom layer S230, which functions to form a second subassembly comprising the top layer, the elastomeric layer, the intermediate substrate, and the bottom layer. Preferably, the bottom layer self-aligns with the intermediate substrate as a result of the bottom layer fitting completely inside a recessed flange on the lower portion of the intermediate layer. The bottom layer is preferably thermally bonded to the intermediate layer. Alternatively, the bottom layer may be bonded to the intermediate layer using adhesive or ultrasonic welding, as shown in FIG. 12G.

Step S250 recites installing the vents of the vent region S250, which functions to permanently form the vents of the vent region. Step S250 is preferably performed by heat staking the vents in place, but may alternatively be performed using adhesive or solvent bonding process. Following step S250, the assembly method 200 may further comprise certain quality control measures, including pressure testing the microfluidic cartridge S252 by blocking all sample and reagent ports, and injecting air into the fluid port, and removing the finished microfluidic cartridge from the second jig S254. Step S260 recites applying labels and packaging, and functions to prepare the microfluidic cartridge with identifying information using at least a barcode label, and preparing the microfluidic cartridge for commercial sale.

An alternative embodiment of an assembly method 300, as shown in FIG. 13, comprises thermally bonding the film layer to the top layer to form a first subassembly S310; adding a vent to the first subassembly and applying a label to create a second subassembly S320; applying an adhesive inside a bottom flange of the intermediate substrate and bonding the bottom layer to the intermediate substrate S330; applying a tag to the intermediate substrate to create a third subassembly S340; positioning the elastomeric layer on the third subassembly to create a fourth subassembly S350; applying adhesive to the fourth subassembly S360; and coupling the second subassembly to the fourth subassembly S370.

The FIGURES illustrate the architecture, functionality and operation of possible implementations of methods according to preferred embodiments, example configurations, and variations thereof. It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose systems that perform the specified functions or acts.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the

preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

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We claim:

- 1. A cartridge, configured to facilitate processing and 5 detecting of a nucleic acid, comprising:
  - a first layer, defining a sample port, a reagent port, a fluid port, and a detection chamber;

an elastomeric layer;

- an intermediate substrate coupled to the first layer, such 10 detecting of a nucleic acid, comprising: that the elastomeric layer is situated between the intermediate substrate and the first layer, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the 15 chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer; and
- a fluidic pathway, wherein the fluidic pathway is fluidically coupled to the sample port, the reagent port, the fluid port, and the detection chamber.
- 2. The cartridge of claim 1
- wherein the fluidic pathway is formed by at least a portion 25 of the first layer and a portion of the elastomeric layer, is configured to be occluded upon manipulation of the elastomeric layer through the set of openings of the corrugated surface, and is configured to transfer a waste fluid to the chamber.
- 3. The cartridge of claim 2, wherein the intermediate substrate is partially separated from the first layer by a film layer.
- 4. The cartridge of claim 2, wherein the chamber of the corrugated surface includes a waste inlet coupled to the 35 fluidic pathway and a waste vent situated at a first side of the fluidic pathway, and wherein the cartridge further comprises a vent region directly opposed to the waste vent at a second side of the fluidic pathway.
- 5. The cartridge of claim 4, wherein a void of the set of 40 voids of the corrugated surface of the chamber receives and positions a magnet proximal the fluidic pathway during operation.
- 6. The cartridge of claim 5, wherein the fluidic pathway comprises a turnabout segment directly adjacent to the void 45 and that crosses the void.
- 7. The cartridge of claim 2, wherein the set of openings of the corrugated surface of the intermediate substrate define a set of valve guides that guide occluding objects toward the elastomeric layer at a set of occlusion positions, during 50 operation.
- 8. The cartridge of claim 1, further comprising a vent region, a region configured to receive a magnet, and a heating region, such that a segment of the fluidic pathway is configured to cross the region configured to receive the 55 magnet and the heating region, and the fluidic pathway is configured to pass through the vent region upstream of the detection chamber.
- 9. The cartridge of claim 8, wherein the heating region is defined by a recessed region of the first layer and spans a 60 long dimension of the first layer.
- 10. The cartridge of claim 1, wherein a terminal portion of the fluidic pathway is coupled to an end vent, configured to provide fine metering of fluid flow.
- 11. The cartridge of claim 1, wherein the detection cham- 65 ber comprises a first, a second, and a third detection chamber segment wherein each of the first, the second, and the third

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detection chamber segment is a broad chamber of which a projection onto a plane is substantially rectangular, wherein a first end of the second detection chamber segment is connected to the first detection chamber segment by a first narrow fluidic channel, and wherein a second end of the second detection chamber segment is connected to the third detection chamber segment by a second narrow fluidic channel.

- 12. A cartridge, configured to facilitate processing and
- a first layer comprising a sample port and a detection chamber:

an elastomeric layer;

- an intermediate substrate including a set of valve guides, wherein the intermediate substrate defines a chamber with a corrugated surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface defines the set of valve guides with a set of openings that provide access to the elastomeric layer; and
- a fluidic pathway, formed by at least a portion of the first layer and a portion of the elastomeric layer, wherein the fluidic pathway is fluidically coupled to the sample port and the detection chamber and comprises a first and second branch extending downstream from a junction, and is configured to be occluded at a set of occlusion positions upon manipulation of the elastomeric layer through the set of valve guides, wherein a first occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the first branch and a second occlusion position of the set of occlusion positions is positioned along the fluidic pathway downstream of the junction and upstream of the second branch, wherein the set of occlusion positions comprises a normally open position and a normally closed position,
- wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation;
- wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that extends toward and abuts the elastomeric layer in preventing fluid bypass at the region;
- wherein a first truncated pathway, including the normally open position and the first branch and excluding the second branch, is defined upon manipulation of the fluidic pathway at the first and second occlusion positions, and
- wherein a second truncated pathway, including the normally closed position and the second branch and excluding the first branch, to the detection chamber is defined upon manipulation of the fluidic pathway at the first and second occlusion positions.
- 13. The cartridge of claim 12, wherein the first layer further comprises a reagent port and a fluid port, and wherein the sample port and the reagent port are configured to couple to a standard pipette tip, and the fluid port is configured to couple to a syringe pump.
- 14. The cartridge of claim 12, wherein the first layer further comprises a vent region including a liquid-imperme-

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able membrane, and wherein the fluidic pathway is configured to pass through the vent region upstream of the detec-

- 15. The cartridge of claim 12, wherein the corrugated surface defines a region configured to receive a magnet 5 providing a magnetic field during operation.
- 16. The cartridge of claim 15, wherein the fluidic pathway comprises a turnabout segment configured to repeatedly cross the region configured to receive a magnet.
- 17. The cartridge of claim 12, wherein a third truncated 10 pathway, coupled to the fluid port, not coupled to the sample port, and not coupled to the reagent port, is defined upon occlusion of the fluidic pathway at a third subset of the set occlusion positions.
- 18. The cartridge of claim 17, wherein the first subset of 15 the set of occlusion positions, the second subset of the set of occlusion positions, and the third subset of the set of occlusion positions are overlapping.
- 19. A cartridge, configured to facilitate processing and detecting nucleic acids, comprising:
  - a shared fluid port configured to receive a processing reagent volume;
  - a first fluidic pathway for processing a first sample;
- a second fluidic pathway for processing a second sample; wherein the first fluidic pathway is coupled to the shared 25 further comprises a vent region and a heating region, such fluid port and the second fluidic pathway is coupled to the shared fluid port, wherein the first fluidic pathway and the second fluidic pathway are each configured to be occluded at a set of occlusion positions, and wherein the set of occlusion positions comprises a normally open position and 30 a normally closed position,
  - wherein the normally open position comprises a first surface of the fluidic pathway at the first layer and a second surface of the fluidic pathway at the elastomeric layer, wherein a void defined between the first surface 35 and the second surface is configured to transition to a closed state upon occlusion by an occluding object applied to the elastomeric layer during operation;
  - wherein the normally closed position is defined by a region of the fluidic pathway, at the first layer that 40 extends toward and abuts the elastomeric layer in preventing fluid bypass at the region; and
  - a first layer, an elastomeric layer, and an intermediate substrate that defines a chamber with a corrugated

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surface directly opposing the first layer, wherein the corrugated surface defines a set of voids external to the chamber and accessible from a direction perpendicular to a broad surface of the first layer, and wherein at least a portion of the corrugated surface includes a set of openings that provide access to the elastomeric layer.

20. The cartridge of claim 19.

- wherein the first layer defines a first sample port-reagent port pair, a second sample port-reagent port pair, the shared fluid port, a first detection chamber, and a second detection chamber,
- wherein the elastomeric layer is situated between the intermediate substrate and the first layer,
- wherein the first fluidic pathway is formed by at least a portion of the first layer and a portion of the elastomeric layer, and is coupled to the first sample port-reagent port pair and to the first detection chamber, and
- wherein the second fluidic pathway is formed by at least a portion of the first layer and a portion of the elastomeric layer, and is coupled to the second sample port-reagent port pair and to the second detection chamber.
- 21. The cartridge of claim 20, wherein the first layer that the first fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the first detection chamber, and the second fluidic pathway is configured to cross the heating region and to pass through the vent region upstream of the second detection chamber.
- 22. The cartridge of claim 19, wherein the corrugated surface of the chamber partially forms a region configured to receive a magnet, which substantially spans a long dimension of the microfluidic cartridge to cross the first fluidic pathway and the second fluidic pathway.
- 23. The cartridge of claim 19, wherein the first fluidic pathway and the second fluidic pathway are identical.
- 24. The cartridge of claim 12, wherein the normally closed position is configured to prevent fluid bypass, and, during operation of the cartridge, is configured to be forced open due to fluid pressure applied by a fluid delivery system.

# EXHIBIT 32







# DR. STEVEN YOUNG VIDEO TESTIMONIAL

NeuMoDx<sup>™</sup> platforms are built to handle large volumes of sample in an automated fashion, integrating the entire molecular diagnostic process from sample to result. Dr. Steven Young, Director of Research and Clinical Trials at TriCore Reference Laboratories, has been working with NeuMoDx<sup>™</sup> on an invitro diagnostics test approved by the FDA, using our instrumentation.

Let Dr. Steven Young share with you the features & benefits of using NeuMoDx™ products in your lab. Watch the video.

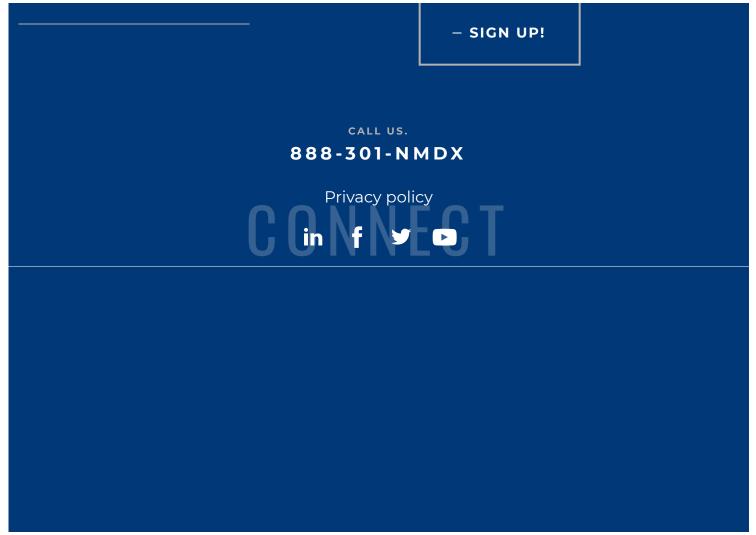
















Available at <a href="http://www.neumodx.com/dr-steven-young-video-testimonial/">http://www.neumodx.com/dr-steven-young-video-testimonial/</a> linking to <a href="https://youtu.be/vukP6gbLBYE">https://youtu.be/vukP6gbLBYE</a> (last visited June 14, 2019)

# EXHIBIT 33

## (12) United States Patent

Williams et al.

## (10) Patent No.: US 10,041,062 B2

#### (45) **Date of Patent:** Aug. 7, 2018

#### (54) SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

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(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35

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- (51) Int. Cl. C12N 15/10 (2006.01) B01L 3/00 (2006.01) (Continued)
- (58) Field of Classification Search
  CPC ....... B29C 66/71; B29C 65/08; B29C 65/606;
  B29C 66/81423; B29C 66/8322;
  (Continued)

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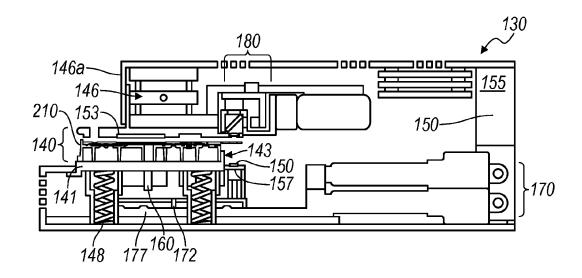
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Primary Examiner — Nathan A Bowers
Assistant Examiner — Lydia Edwards
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#### (57) ABSTRACT

A system and method for processing and detecting nucleic acids from a set of biological samples, comprising: a capture plate and a capture plate module configured to facilitate binding of nucleic acids within the set of biological samples to magnetic beads; a molecular diagnostic module configured to receive nucleic acids bound to magnetic beads, isolate nucleic acids, and analyze nucleic acids, comprising a cartridge receiving module, a heating/cooling subsystem and a magnet configured to facilitate isolation of nucleic acids, a valve actuation subsystem configured to control fluid flow through a microfluidic cartridge for processing nucleic acids, and an optical subsystem for analysis of nucleic acids; a fluid handling system configured to deliver samples and reagents to components of the system to facilitate molecular diagnostic protocols; and an assay strip configured to combine nucleic acid samples with molecular diagnostic reagents for analysis of nucleic acids.

#### 20 Claims, 24 Drawing Sheets



#### US 10,041,062 B2

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#### Related U.S. Application Data

continuation of application No. 13/766,359, filed on Feb. 13, 2013, now Pat. No. 9,050,594, and a continuation of application No. 13/765,996, filed on Feb. 13, 2013, now Pat. No. 9,738,887.

(60) Provisional application No. 61/667,606, filed on Jul. 3, 2012, provisional application No. 61/598,240, filed on Feb. 13, 2012.

(51) Int. Cl. C12M 3/00 (2006.01)C12Q 1/68 (2018.01)B01L 7/00 (2006.01)C12Q 1/686 (2018.01)B01L 9/00 (2006.01)C12Q 1/6806 (2018.01)C12Q 1/6813 (2018.01)C12N 13/00 (2006.01)B29C 65/08 (2006.01)B29C 65/60 (2006.01)B29C 65/00 (2006.01)B29L 31/00 (2006.01)B29C 65/48 (2006.01)

(52) U.S. Cl.

CPC ....... B01L 3/5027 (2013.01); B01L 3/50273 (2013.01); B01L 3/502707 (2013.01); B01L 3/502723 (2013.01); B01L 3/502738 (2013.01); **B01L** 3/502761 (2013.01); **B01L** 3/527 (2013.01); B01L 3/567 (2013.01); B01L 7/52 (2013.01); B01L 7/525 (2013.01); B01L 9/00 (2013.01); C12M 23/42 (2013.01); C12Q 1/68 (2013.01); C12Q 1/6806 (2013.01); C12Q 1/686 (2013.01); C12Q 1/6813 (2013.01); B01L 3/5025 (2013.01); B01L 3/502746 (2013.01); B01L 2200/025 (2013.01); B01L 2200/0605 (2013.01); B01L 2200/0615 (2013.01); B01L 2200/0668 (2013.01); B01L 2200/0684 (2013.01); B01L 2200/0689 (2013.01); B01L 2200/10 (2013.01); B01L 2200/12 (2013.01); B01L 2200/142 (2013.01); B01L 2300/021 (2013.01); B01L 2300/022 (2013.01); B01L 2300/044 (2013.01); B01L 2300/06 (2013.01); B01L 2300/0609 (2013.01); B01L 2300/0627 (2013.01); B01L 2300/0636 (2013.01); B01L 2300/0672 (2013.01); B01L 2300/0681 (2013.01); B01L 2300/087 (2013.01); B01L 2300/0809 (2013.01); B01L 2300/0816 (2013.01); B01L 2300/0861 (2013.01); B01L 2300/0864 (2013.01); B01L 2300/0867 (2013.01); B01L 2300/0883 (2013.01); B01L 2300/0887 (2013.01); B01L 2300/0893 (2013.01); B01L 2300/123 (2013.01); B01L 2300/14 (2013.01); B01L 2300/16 (2013.01); B01L 2300/1822 (2013.01); B01L 2300/1827 (2013.01); B01L 2400/043 (2013.01); B01L 2400/0406 (2013.01); B01L 2400/0478 (2013.01); B01L 2400/0481 (2013.01); B01L 2400/0487 (2013.01); B01L 2400/065 (2013.01); B01L 2400/0622 (2013.01); B01L 2400/0655 (2013.01); B01L 2400/0694 (2013.01); B01L 2400/086 (2013.01); B29C 65/08 (2013.01); B29C 65/484 (2013.01); B29C 65/606 (2013.01); B29C 66/71

(2013.01); B29C 66/81423 (2013.01); B29C 66/8322 (2013.01); B29L 2031/756 (2013.01); C12N 13/00 (2013.01)

#### 58) Field of Classification Search

CPC ........ B29K 2021/00; B01L 2200/0684; B01L 2200/0689; B01L 2200/10; B01L 2300/1827; B01L 2400/0487; B01L 2400/0655; B01L 3/5027

See application file for complete search history.

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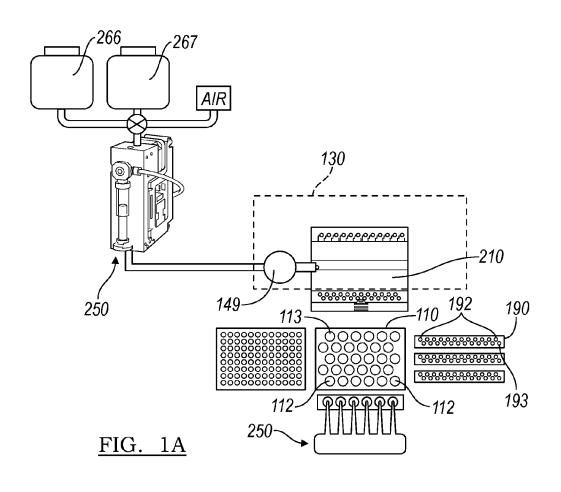
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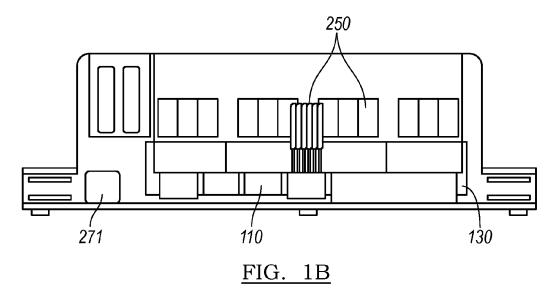
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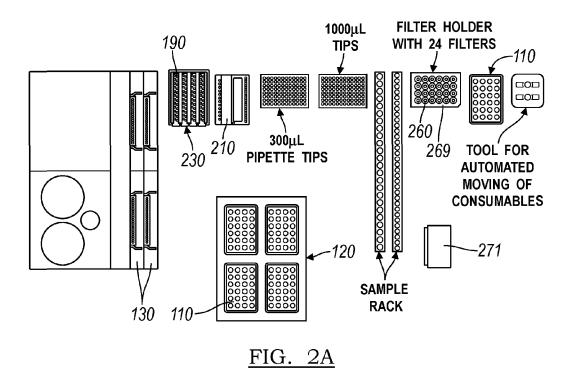
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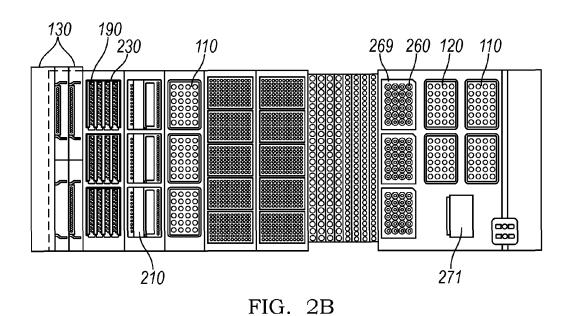




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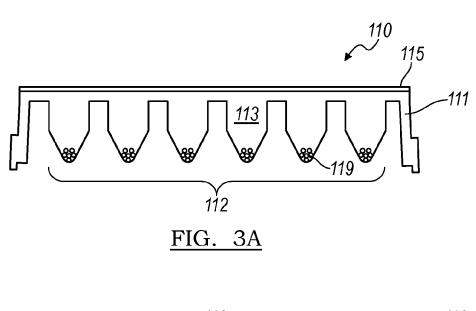
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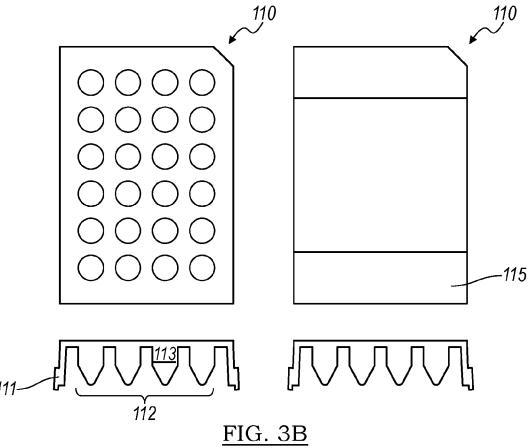




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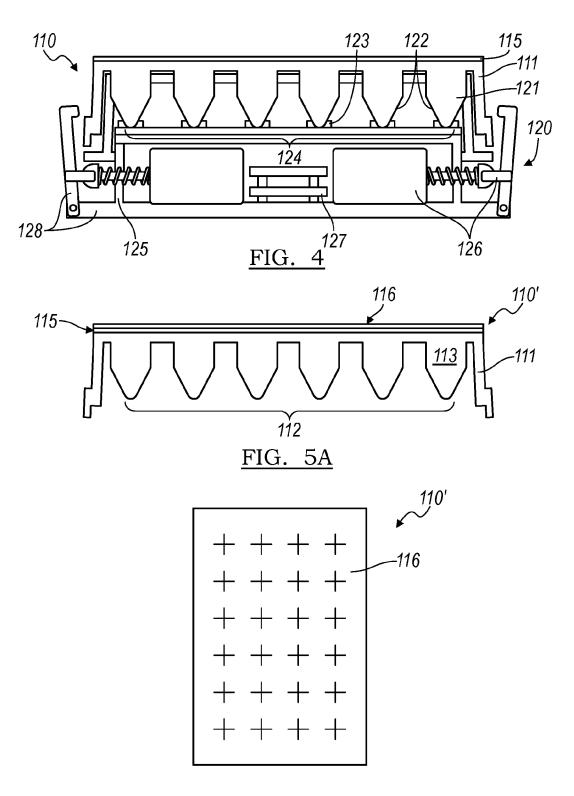
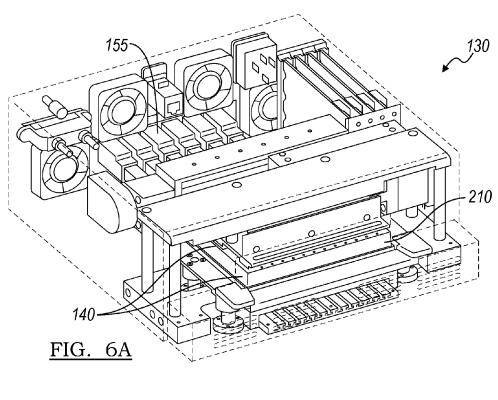
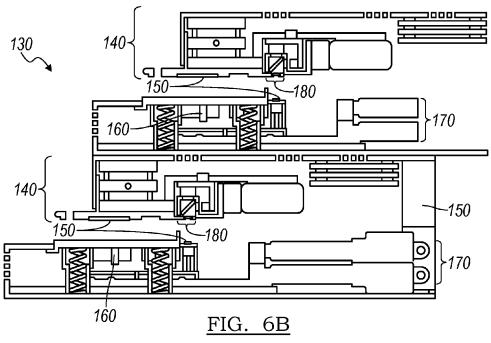


FIG. 5B

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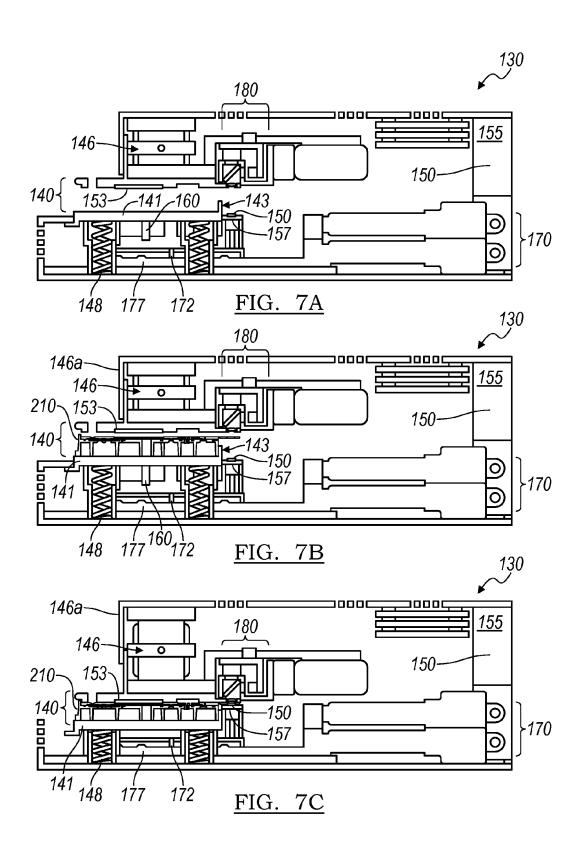
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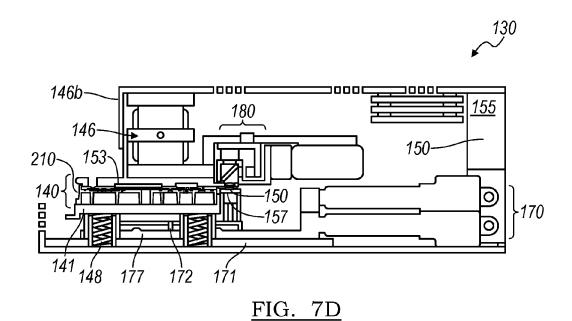
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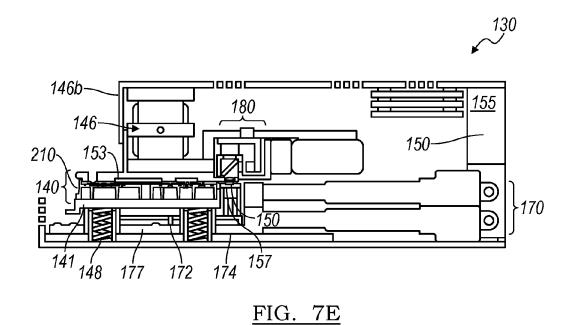
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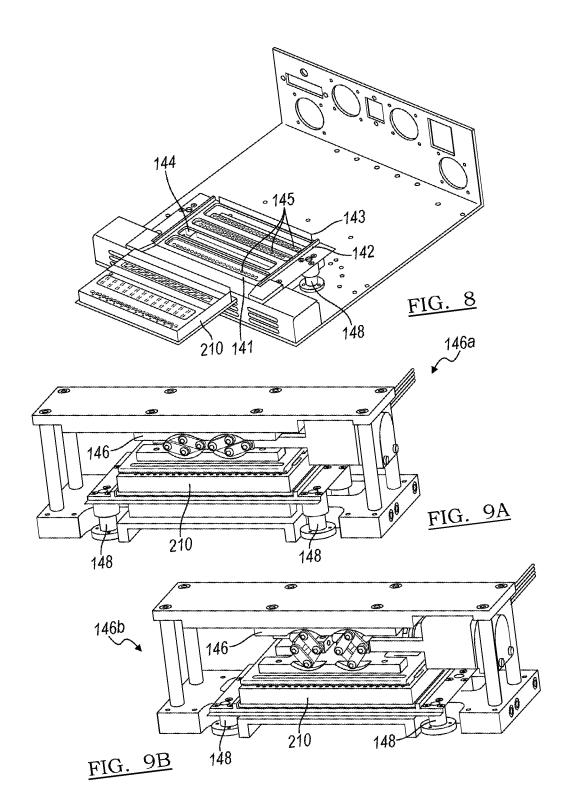


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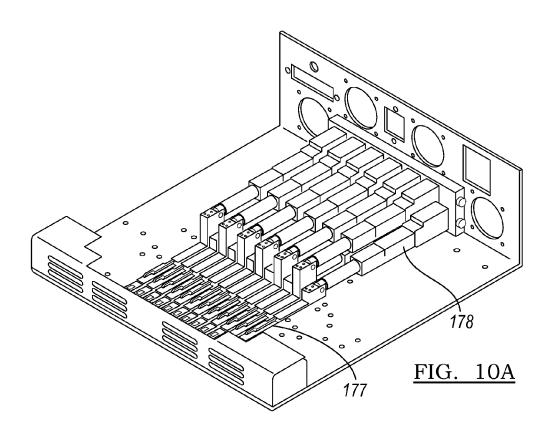


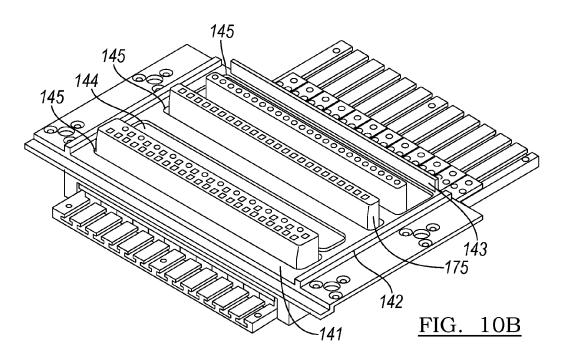




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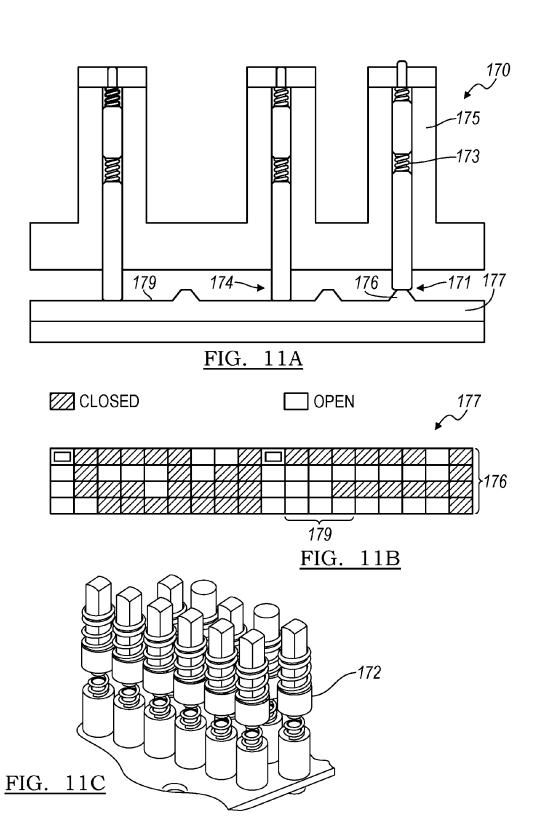
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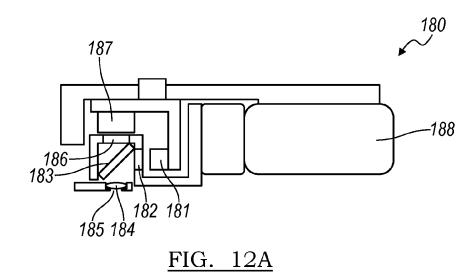
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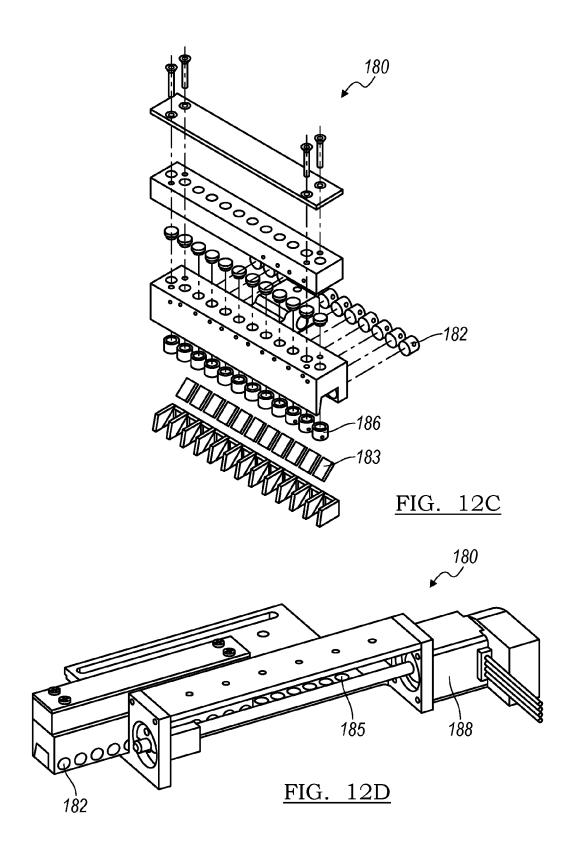


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FIG. 12B

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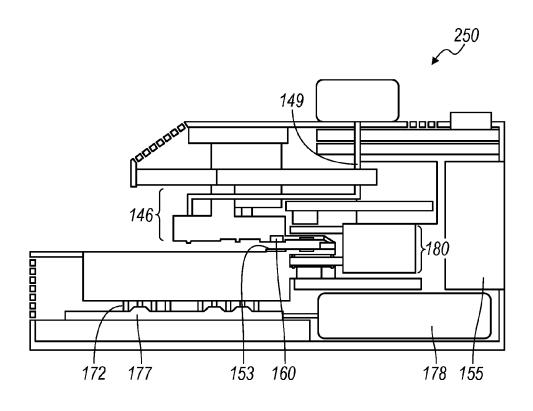
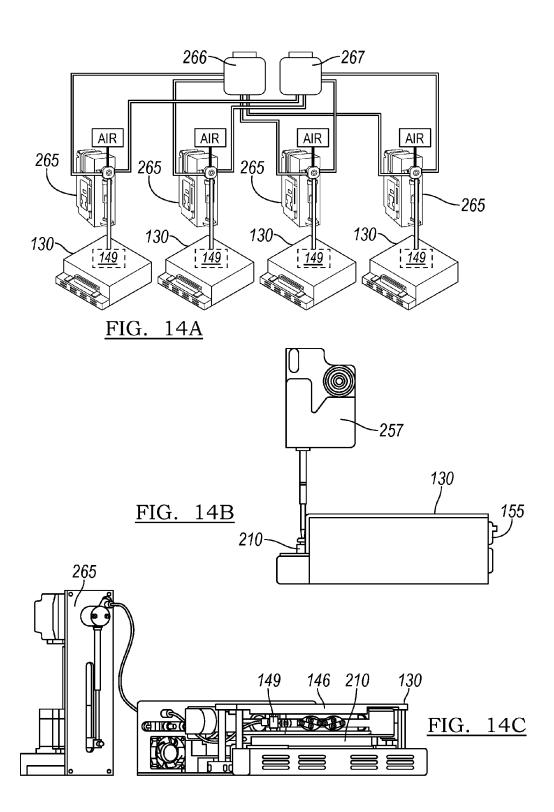


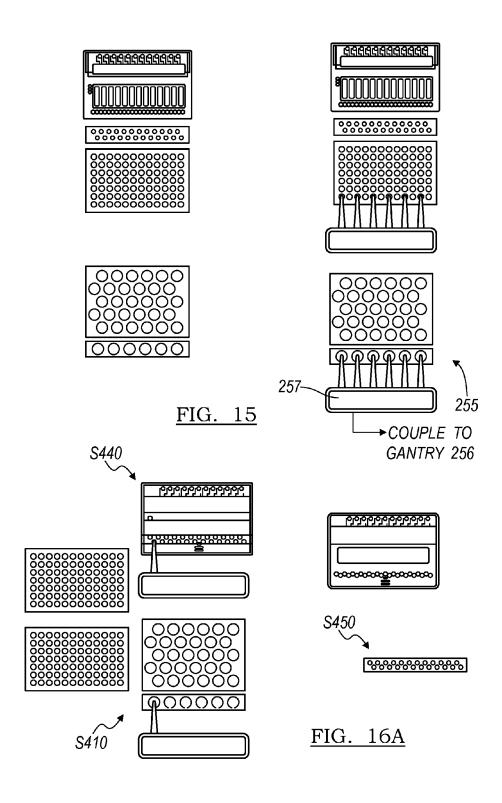
FIG. 13

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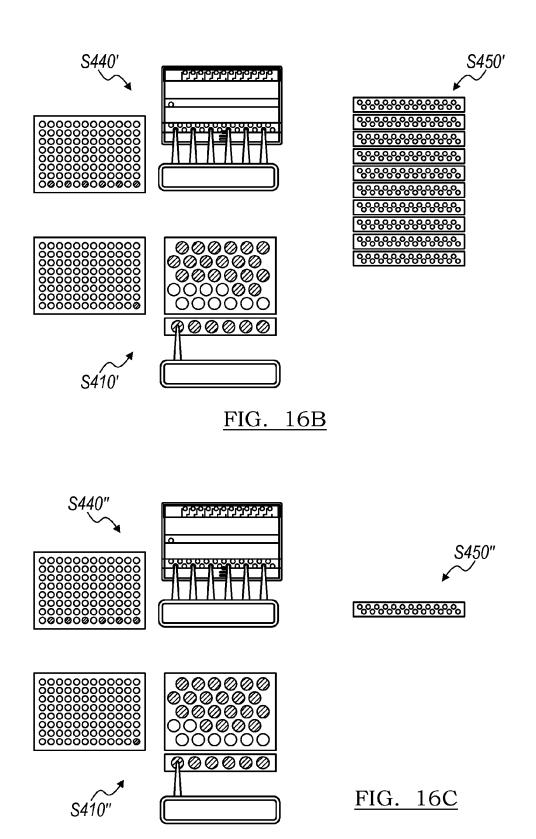


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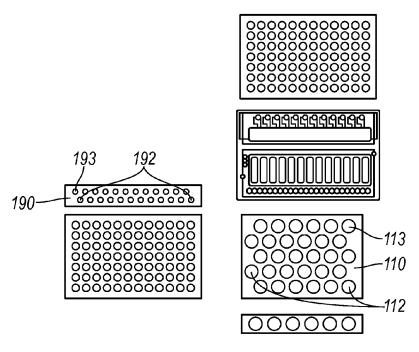
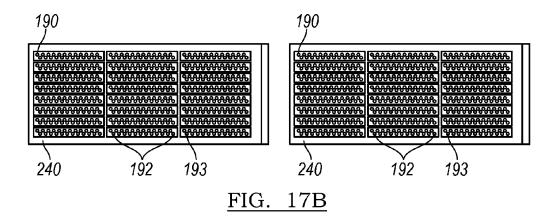
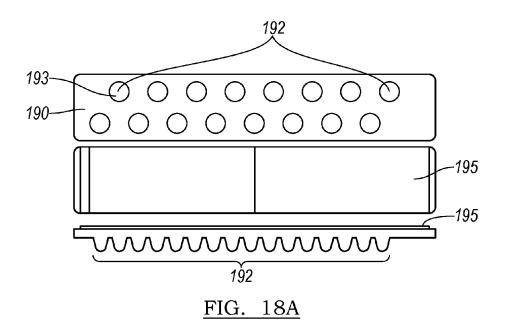


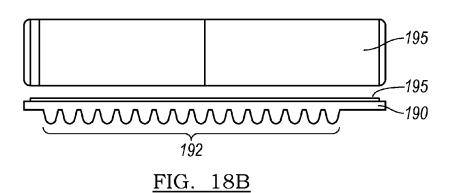
FIG. 17A



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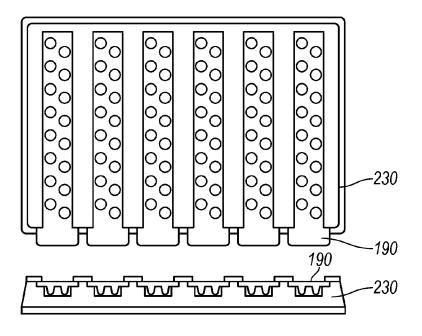
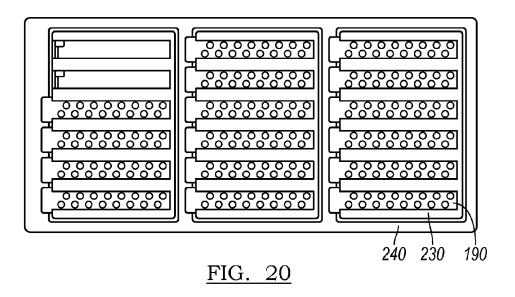


FIG. 19



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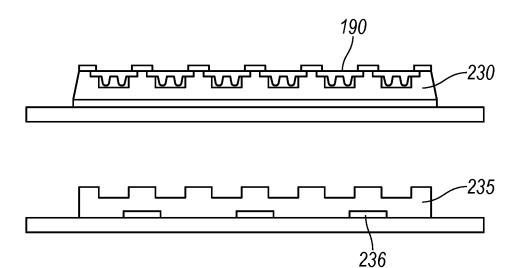


FIG. 21A

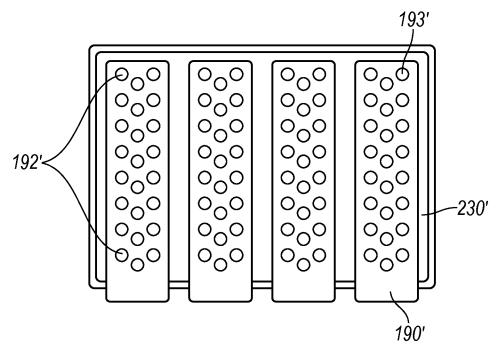
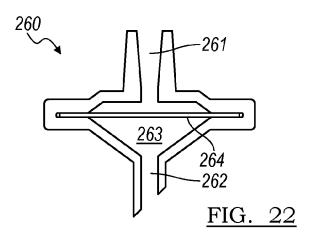
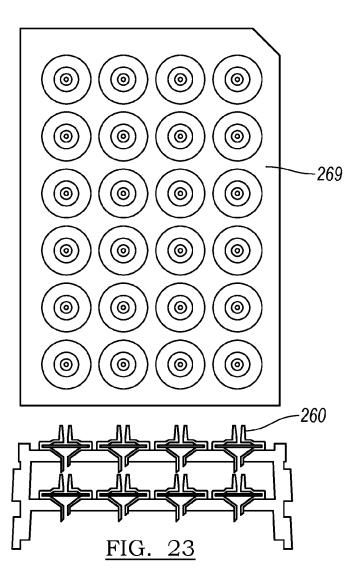


FIG. 21B

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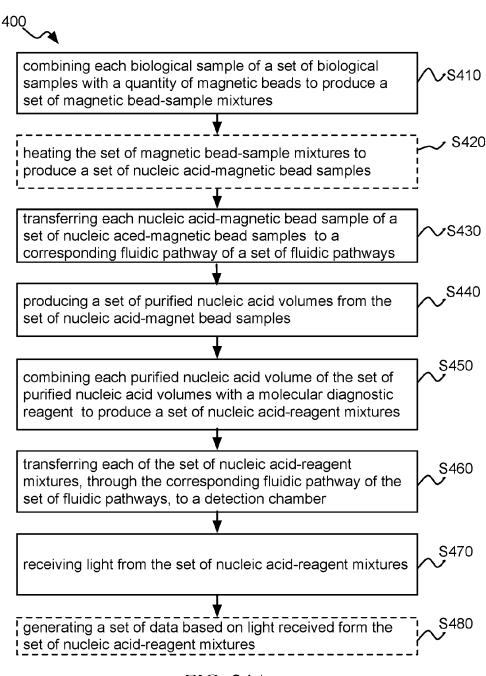


FIG. 24A

U.S. Patent Aug. 7, 2018 **Sheet 23 of 24** US 10,041,062 B2 transferring each nucleic acid-magnetic bead sample of a **/**\$430 set of nucleic aced-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways S432 occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions to define at least one truncated fluidic pathway FIG. 24B S440 producing a set of purified nucleic acid volumes from the set of nucleic acid-magnet bead samples providing a magnetic field S441, such that each fluidic S441 pathway in the set of fluidic pathways is configured to cross the magnetic field S442 providing a heater configured to span a heating region of the set of fluidic pathways **S443** occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions to define at least one truncated fluidic pathway containing a nucleic acid-magnet bead sample and coupled to a source for delivery of a wash solution and a release solution delivering a wash solution through a portion of at least one fluidic pathway

FIG. 24C

S445

delivering a release solution through a portion of at least

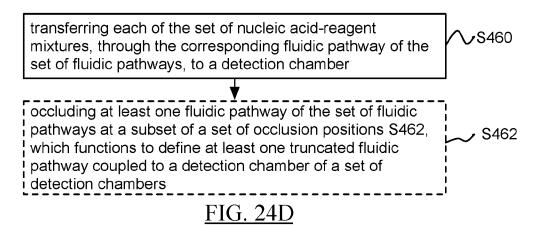
one fluidic pathway

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#### 1

# SYSTEM AND METHOD FOR PROCESSING AND DETECTING NUCLEIC ACIDS

## CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of co-pending U.S. patent application Ser. No. 14/704,215, filed 5 May 2015, which is a continuation of co-pending U.S. patent application Ser. No. 13/766,359, filed 13 Feb. 2013, which claims the benefit of U.S. Provisional Application Ser. No. 61/667, 606, filed on 3 Jul. 2012, and U.S. Provisional Application Ser. No. 61/598,240, filed on 13 Feb. 2012, which are incorporated herein in their entirety by this reference. This application is also a continuation of co-pending U.S. application Ser. No. 13/765,996, filed 13 Feb. 2013, which is incorporated herein in its entirety by this reference.

#### TECHNICAL FIELD

This invention relates generally to the molecular diagnostics field, and more specifically to an improved system and method for processing and detecting nucleic acids.

#### BACKGROUND

Molecular diagnostics is a clinical laboratory discipline that has developed rapidly during the last 25 years. It originated from basic biochemistry and molecular biology 30 research procedures, but now has become an independent discipline focused on routine analysis of nucleic acids (NA), including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) for diagnostic use in healthcare and other fields involving analysis of nucleic acids. Molecular diagnostic 35 analysis of biological samples can include the detection of one or more nucleic acid materials present in the specimen. The particular analysis performed may be qualitative and/or quantitative. Methods of analysis typically involve isolation, purification, and amplification of nucleic acid materials, and 40 polymerase chain reaction (PCR) is a common technique used to amplify nucleic acids. Often, a nucleic acid sample to be analyzed is obtained in insufficient quantity, quality, and/or purity, hindering a robust implementation of a diagnostic technique. Current sample processing methods and 45 molecular diagnostic techniques are often labor/time intensive, low throughput, and expensive, and systems of analysis are insufficient. Furthermore, methods of isolation, processing, and amplification are specific to certain sample matrices and/or nucleic acid types and not applicable across common 50 sample and nucleic acid types.

Due to these and other deficiencies of current molecular diagnostic systems and methods, there is thus a need for and improved system and method for processing and detecting nucleic acids. This invention provides such a system and 55 method.

#### BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1A-1B depict an embodiment of a system for 60 processing and detecting nucleic acids;

FIGS. 2A-2B depict an embodiment of elements, and a top view of an embodiment of a system worktable, respectively, of an embodiment of a system for processing and detecting nucleic acids;

FIGS. 3A-3B depict an embodiment of a capture plate for combining a sample with magnetic beads;

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FIG. 4 depicts an embodiment of a capture plate module to facilitate lysis of a biological sample and combination of the biological sample with magnetic beads;

FIGS. 5A-5B depict an alternative embodiment of a capture plate;

FIGS. 6A-6B depict embodiments of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 7A-7E depict a sequence of operations performed by elements of an embodiment of a molecular diagnostic module;

FIG. 8 depicts an embodiment of a microfluidic cartridge and an embodiment of a cartridge platform;

FIGS. 9A-9B depict configurations of a linear actuator of an embodiment of a molecular diagnostic module;

FIGS. 10A-10B depict elements of an embodiment of a valve actuation subsystem of a molecular diagnostic module:

FIGS. 11A-11C depict an embodiment of a valve actuation subsystem of a molecular diagnostic module;

FIGS. 12A-12D depict elements of an embodiment of an optical subsystem of a molecular diagnostic module;

FIG. 13 depicts a side view of an alternative embodiment of a molecular diagnostic module for processing and detecting nucleic acids;

FIGS. 14A-14C depict an embodiment of a fluid handling system of a system for processing and detecting nucleic acids:

FIG. 15 depicts embodiments of elements of the fluid handling system;

FIGS. 16A-16C are schematics depicting example methods for processing and detecting nucleic acids;

FIGS. 17A-17B show embodiments of consumables and reagents used in a system for processing and detecting nucleic acids;

FIGS. 18A-18B depict an embodiment of an assay strip to facilitate analysis of a sample containing nucleic acids;

FIG. 19 depicts an embodiment of an assay strip holder; FIG. 20 depicts an embodiment of an assay strip carrier; FIGS. 21A-21B show alternative embodiments of assay strip holders and assay strips, respectively;

FIG. 22 shows an embodiment of a filter to facilitate processing and detecting of nucleic acids;

FIG. 23 shows an embodiment of a filter holder to facilitate processing and detecting of nucleic acids; and

FIGS. **24**A-**24**D depict embodiments of a method for processing and detecting nucleic acids.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following description of preferred embodiments of the invention is not intended to limit the invention to these preferred embodiments, but rather to enable any person skilled in the art to make and use this invention.

1. System for Processing and Detecting Nucleic Acids

As shown in FIGS. 1A-1B and 7A, an embodiment of a system 100 for processing and detecting nucleic acids comprises: a capture plate 110 configured to facilitate binding of nucleic acids within a biological sample to a set of magnetic beads 119; a molecular diagnostic module 130 comprising a microfluidic cartridge receiving module 140, heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, an optical subsystem 180; and an assay strip 190 configured to facilitate mixing of molecular diagnostic reagents with a nucleic acid volume. Other embodiments of the system 100 may further comprise at least one of a capture plate module 120 configured to support the capture

plate 110; a filter 200 and filter holder 205 to facilitate sample preparation; a microfluidic cartridge 210 configured to facilitate sample processing; an assay strip holder 230; an

to facilitate sample processing; an assay strip holder 230; an assay strip carrier 240; a liquid handling system 250 configured to facilitate gas and fluid delivery to different ele- 5 ments of the system 100; a processor configured to analyze data resulting from a run of the system 100; and a user interface configured to allow a user to interact with the system 100. The system 100 thus functions to receive biological samples containing nucleic acids (i.e., impure 10 nucleic acid samples), separate nucleic acids from the biological samples, and analyze nucleic acid samples according to at least one molecular diagnostic protocol (e.g., PCR). Preferably, the system 100 is a walkaway system by which a user loads a set of biological samples containing nucleic 15 acids, and receives a set of data resulting from a molecular diagnostic protocol without any further sample manipulation by the user. Alternatively, the system 100 facilitates aspects of sample preparation for a molecular diagnostic protocol,

with some sample manipulation performed by the user.

In one example workflow of the system 100, a liquid handling system 250 aspirates a set of biological samples containing nucleic acids (i.e., impure nucleic acid samples), and dispenses the set of biological samples into a capture plate 110 to be lysed and combined with magnetic beads 25 (containing a proprietary affinity coating to bind the nucleic acids to the magnetic beads) by a capture plate module 120. The liquid handling system 250 then aspirates substantially all of each sample of the set of lysed biological samples combined with magnetic beads (i.e., set of magnetic beadsamples) from the capture plate 110, and dispenses the set of magnetic bead-samples into a microfluidic cartridge 210, aligned within a cartridge receiving module 140 of a molecular diagnostic module 130, and configured to be manipulated by the molecular diagnostic module 130. A 35 heating and cooling subsystem 150, a magnet 160, and a valve actuation subsystem 170 of the molecular diagnostic module 130 then facilitate separation of a set of nucleic acids from the magnetic bead-samples, as the liquid handling system 250 dispenses wash solutions, release solutions, 40 and/or air at appropriate stages. The liquid handling system 250 then aspirates the set of nucleic acids from the microfluidic cartridge 210 contained within the molecular diagnostic module 130, combines the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, 45 and dispenses the set of nucleic acids combined with the set of molecular diagnostic reagents (i.e., set of nucleic acidreagent mixtures) into the microfluidic cartridge 210 within the molecular diagnostic module 130. The detection chamber heaters 157, optical subsystem 180 and valve actuation 50 subsystem 170 of the molecular diagnostic module 130 then facilitate analysis of the set of nucleic acid-reagent mixtures by a processor configured to display information on a user interface.

As stated, the above workflow is just one example workflow of the system 100, and other workflows of the system 100 and methods of processing and detecting nucleic acid samples are further described in Section 2 below. A detailed description of elements of an embodiment of the system 100 are described in sections 1.1-1.6 below.

1.1 System—Capture Plate and Capture Plate Module

As shown in FIGS. 3A and 3B, the capture plate 110 comprises a capture plate substrate 111 comprising a set of wells 112 and a puncturable foil seal 115, and functions to facilitate binding of nucleic acids within a biological sample 65 to a set of magnetic beads 119. Preferably, the entire capture plate 110 is configured to be a consumable (i.e., disposable),

such that each well of the capture plate 110 can only be used once yet the remaining unused wells can be used during additional runs of the system 100. Alternatively, at least a portion of the capture plate 110 is configured to be reusable, such that additional mixing or reagent additions can be

such that additional mixing or reagent additions can be performed and portions of the capture plate 110 may be used for multiple runs of the system 100. In one variation of the capture plate 110, the capture plate substrate 111 is reusable, while the puncturable foil seal 115 is disposable and replaced after each run of the system 100.

The capture plate substrate 111 is configured such that the capture plate 110 is capable of resting on a flat surface, can be stacked with another capture plate 110, and also can be manipulated with industry standard instrument components for handling of microtiter plates. The capture plate substrate also functions to define the set of wells 112 and to couple to the puncturable foil seal 115. The capture plate substrate 111 is preferably composed of a PCR-compatible polymer that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 112 of the capture plate substrate 111 function to receive at least one biological sample which contain or are suspected of potentially containing nucleic acids, and to facilitate combination of the biological sample with a set of magnetic beads 119. Preferably, the wells 113 are each configured to accommodate not only a biological sample, but also to facilitate mixing of the biological sample with a set of magnetic beads 119 (e.g., using a pipettor, the liquid handling system 250 or other apparatus), which preferably are preloaded in wells 112, or alternatively may be added by an operator. Preferably, the wells are also deeper than they are wide to allow a significant number of wells 112 (e.g. 24) with a clinically relevant sample volumes, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wider than they are deep to facilitate larger devices for mixing the biological samples with the magnetic beads 119. Each well 113 of the set of wells 112 also preferably has a conically shaped bottom region, as shown in FIG. 3A, to facilitate complete aspiration of a fluid from a well. Alternatively, each well 113 may not have a conically shaped bottom region. Additionally, in the orientation shown in FIG. 3A, the tops of each well 113 in the set of wells 112 preferably form raised edges protruding from the capture plate substrate 111, in order to facilitate sealing of each well 113 by the puncturable foil seal 115. Alternatively, the tops of each well 113 in the set of wells 112 may not form raised edges protruding from the capture plate substrate ill. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. However, the magnetic beads may alternatively be any appropriate magnetic beads (e.g. magnetic, parmagnetic, or superparamagnetic) configured to facilitate biomagnetic separation.

Each quantity of magnetic beads 119 may be accompanied by lysing reagents (e.g. proteinase K) and a sample
process control comprising nucleic acid sequences for DNA
and RNA, which function to lyse biological samples and to
provide a mechanism by which sample process controls may
be later detected to verify processing fidelity and assay
accuracy. The sample process control comprising nucleic
acid sequences for DNA and RNA allows one version of the
capture plate to facilitate assays involving DNA and RNA

detection. Preferably, the quantity of magnetic beads 119, lysing reagents, and sample process controls is dried within each well to improve shelf life; however, the quantity of magnetic beads 119, lysing reagents, and sample process controls may alternatively be in liquid form.

The puncturable foil seal 115 functions to isolate each well 113 of the set of wells 112, prevent contamination of the contents of each of the set of wells 112, protect the magnetic beads 119 and other reagents stored in wells 112 from degradation, and provide information identifying the capture 10 plate 110. The puncturable foil seal 115 preferably seals each well 113 of the capture plate 110, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one variation, the puncturable foil seal 115 also forms a seal 15 around an element that punctures it, and in another variation, the puncturable foil seal 115 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 115 is also preferably labeled with identifying information including at least one of manufac- 20 turer information, capture plate contents, the lot of the contents, an expiry date, and a unique electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal 115 does not extend beyond puncturable foil seal 115 may be any appropriate size and/or include protruding features (e.g., tabs) that facilitate handling of the capture plate.

In one variation, the capture plate 110 may be prepackaged at least with magnetic beads 119, such that each well 30 113 in the set of wells 112 is prepackaged with a set of magnetic beads 119 defined by a specific quantity or concentration of magnetic beads. The set of wells 112 may then be sealed by the puncturable foil seal 115, which is configured to be punctured by an external element that delivers 35 volumes of biological samples to be mixed with the magnetic beads 119. In another variation, the capture plate 110 may not be prepackaged with magnetic beads 119, but the wells 113 of the capture plate may still be sealed with a puncturable foil seal 115. In this variation, the puncturable 40 foil seal 115 is configured to be punctured by at least one external element, for co-delivery of biological samples and magnetic beads intended to be combined.

A variation of the capture plate 110' may further comprise a slotted rubber membrane 116, as shown in FIGS. 5A and 45 5B, configured to provide access through the puncturable foil seal 115 to the set of wells 112. The slotted rubber membrane 116 thus functions to prevent or reduce splashing, evaporation, and/or aerosolization of contents of the set of wells 112. Preferably, the slotted rubber membrane 116 50 comprises slots that are self-sealing and centered over wells of the set of wells 112, and further does not extend beyond the footprint of the capture plate 110. Alternatively, the slots of the slotted rubber membrane 116 may not be self-sealing, and/or the slotted rubber membrane 116 may be any appro- 55 priate size and comprise features that extend beyond the footprint of the capture plate 110.

In a specific example, the capture plate 110 comprises 24 wells 113 with an 18 mm center-to-center pitch, each well having a volumetric capacity of 2 mL, and is compliant with 60 Society for Laboratory Automation and Screening (SLAS) standards. Each well 113 of the capture plate 110 in the specific example is also prepackaged with a specified quantity of magnetic beads 119, and comprises a protruding top edge that is heat sealed to a puncturable foil seal. In addition, 65 each well 113 also contains other reagents beneficial for processing and monitoring the sample, including proteinase

K and one or more specific nucleic acid stands designed to serve as a process control. The specific example of the capture plate 110 can thus combine two groups of 12 biological samples with magnetic beads. The capture plate 110 in the specific example is produced by injection mold-

ing, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier.

An embodiment of the system 100 may further comprise a capture plate module 120, as shown in FIG. 4, which functions to receive, support, and heat a capture plate 110. The capture plate module 120 preferably comprises a thermally conducting substrate 121 configured to cradle a capture plate 110, a capture plate heater 123, a capture plate receiving module 125, and a capture plate electronics module 127. Preferably, the capture plate module 120 functions to facilitate lysis of a biological sample deposited into a well 113 of the capture plate, and to facilitate binding of nucleic acids (i.e., within a lysed biological sample) to a quantity of magnetic beads 119 within a well 113 of the capture plate 110. In a specific example, the capture plate module 120 has

dimensions of 108 mm×156 mm×45 mm and is configured

to rest on a flat surface.

The thermally conducting substrate 121 is configured to the footprint of the capture plate 110, but alternatively, the 25 cradle and support the capture plate 110, and functions to conduct heat to the set of wells 112 of the capture plate 110. Preferably, the thermally conducting substrate 121 is also configured to reversibly couple to the capture plate 110, and comprises a set of indentations 122 that encircle each well 113 in the set of wells 112. In one variation, the indentations 122 completely conform to the external surface of each well 113 of the capture plate 110, but in another variation, the indentations 122 may encircle a portion of each well 113 of the capture plate 110. Additionally, the indentations 122 are preferably thermally conducting in order to conduct heat to the set of wells 112, and portions of the thermally conducting substrate 121 aside from the indentations 122 are composed of non-conducting, rigid material. Alternatively, the entire thermally conducting substrate 121 may be composed of a material that is thermally conducting.

The capture plate heater 123 is preferably coupled to the thermally conducting substrate 121, and functions to transfer heat, through the thermally conducting substrate 121, to a well 113 of the capture plate 110. The capture plate heater 123 preferably conforms to at least a portion of an indentation 122 of the thermally conducting substrate 121, to facilitate heat transfer through the indentation 122 to an individual well 113 of the capture plate 110. In this variation, the capture plate heater 123 is one of a set of capture plate heaters 124, wherein each capture plate heater 123 in the set of capture plate heaters 124 transfers heat to an individual well 113 of the set of wells 112 of the capture plate 110. Alternatively, the capture plate heater 123 may conform to portions of multiple indentations 122 of the thermally conducting substrate 121, such that the capture plate heater 123 is configured to transfer heat to multiple wells 113 of the capture plate 110. Preferably, the capture plate heater 123 is a resistance heater, but alternatively, the capture plate heater 123 may be a Peltier or any appropriate heater configured to transfer heat to the capture plate 110. The capture plate heater 123 may also further couple to a heat sink.

The capture plate receiving module 125 comprises a capture plate actuation system 126 that functions to couple the capture plate module 120 to a capture plate 110. As shown in FIG. 4, the capture plate actuation system 126 comprises a structural support with hinged grips 128 and at least one capture plate module actuator 129. The capture

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plate module actuator 129 is preferably a push-type solenoid with a spring return, but may alternatively be any appropriate linear actuator, such as a hydraulic actuator. The structural support with hinged grips 128 preferably couples to the capture plate heater 123 and houses the capture plate module 5 actuator 129, such that, in a first configuration, actuation of the capture plate module actuator 129 outwardly displaces the hinged grips (allowing the capture plate module 120 to receive a capture plate 110), and in a second configuration, actuation of the capture plate module actuator 129 inwardly 10 displaces the hinged grips (allowing the capture plate module 120 to couple to the capture plate 110). The structural support with hinged grips 128 may further comprise a textured and/or high-friction surface configured to grip a capture plate 110, but alternatively may not comprise a 15 textured and/or high-friction surface.

The capture plate electronics module 127 is coupled to the capture plate heater 123 and the capture plate actuation system 126, and functions to enable control of the capture plate heater 123 and the capture plate actuation system 126. 20 Preferably, the capture plate electronics module 127 modulates an output of the capture plate heater 123, in order to controllably heat at least one well 113 of the capture plate 110. Additionally, the capture plate electronics module 127 preferably modulates the capture plate actuation system 126, 25 in order to controllably couple the capture plate module 120 to a capture plate 110. Preferably, the capture plate electronics module 127 is coupled to an external power supply, such that the capture plate module 120 does not include an integrated power supply; however, in alternative embodi- 30 ments, the capture plate electronics module 127 may be coupled to a power supply integrated with the capture plate module 120.

#### 1.2 System—Molecular Diagnostic Module

As shown in FIGS. 6A and 6B, an embodiment of the 35 molecular diagnostic module 130 of the system 100 includes a cartridge receiving module 140, a heating and cooling subsystem 150, a magnet 160, a valve actuation subsystem 170, and an optical subsystem 180, and functions to manipulate a microfluidic cartridge 210 for processing of a biologi- 40 cal sample containing nucleic acids. The molecular diagnostic module 130 is preferably configured to operate in parallel with at least one other molecular diagnostic module 130, such that multiple microfluidic cartridges 210 containing biological samples may be processed simultaneously. In 45 a first variation, the molecular diagnostic module 130 is configured to be stackable with another molecular diagnostic module 130 in a manner that enables access to a microfluidic cartridge 210 within each molecular diagnostic module 130; an example of the first variation is shown in FIG. 6B, where 50 the molecular diagnostic modules 130 are stacked in a staggered configuration. In the first variation, each molecular diagnostic module 130 may further comprise locking pins or other appropriate mechanisms to couple the stacked molecular diagnostic modules 130 together. In another 55 variation, the molecular diagnostic module 130 may not be configured to stack with another molecular diagnostic module, such that the molecular diagnostic modules 130 are configured to rest side-by-side on the same plane. Elements of an embodiment of the molecular diagnostic module 130 60 are further described in sections 1.2.1 to 1.2.5 below.

1.2.1 Molecular Diagnostic Module—Cartridge Receiving Module

As shown in FIG. 0.4, the contridge receiving module 140.

As shown in FIG. 9A, the cartridge receiving module 140 of the molecular diagnostic module 130 comprises a cartridge platform 141 including a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and

a set of valve actuation slots 145; a linear actuator 146 configured to displace a microfluidic cartridge 210 resting on the cartridge platform 141; and a set of springs 148 coupled to the cartridge platform 141. The cartridge receiving module 140 thus functions to receive, align, and compress a microfluidic cartridge 210 for processing of a biological sample according to a molecular diagnostic assay protocol. As shown in FIGS. 7A-7C, the cartridge platform 141 is preferably configured to receive a microfluidic cartridge 210 along a cartridge loading guiderail 142 until it reaches a cartridge stop 143, and be vertically displaced by the linear actuator 146, which places a biasing force against the set of springs 148 coupled to the cartridge platform 141. The magnet receiving slot 144 and the set of valve actuation slots 145 provide access, by a magnet 160 and a valve actuation subsystem 170, to the microfluidic cartridge 210, as the microfluidic cartridge is vertically displaced by the linear actuator 146.

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The cartridge platform 141 includes a cartridge loading guiderail 142, a cartridge stop 143, a magnet receiving slot 144, and a set of valve actuation slots 145, and functions to receive and align a microfluidic cartridge 210, while providing access to the microfluidic cartridge 210 by a magnet 160 and a valve actuation subsystem 170. As shown in FIG. 8, an embodiment of the cartridge platform 141 includes a pair of parallel cartridge loading guiderails 142, initiating at a pair of inwardly tapering protrusions configured to guide a microfluidic cartridge toward the pair of parallel cartridge loading guiderails 142, and spanning two short edges of the cartridge platform 141. The embodiment of the cartridge platform 141 also includes a cartridge stop 143 comprising a vertical tab oriented perpendicular to the cartridge loading guiderails 142, and spanning a long edge of the cartridge platform. Preferably, the cartridge loading guiderails 142 and the cartridge stop 143 are configured such that a microfluidic cartridge 210 slides between the cartridge loading guiderails 142 and hits the cartridge stop 143 to signal proper alignment. Alternatively, the cartridge loading guiderails 142 and the cartridge stop 143 may be configured such that a microfluidic cartridge slides over or along the cartridge loading guiderails 142, after which the cartridge stop 143 couples to a portion of the microfluidic cartridge 210 to ensure proper alignment of the microfluidic cartridge. Additional variations of the cartridge loading guiderails 142 and the cartridge stop 143 may be used to enable reception and alignment of a microfluidic cartridge 210 by the molecular diagnostic module 130, and are known by those skilled in

The embodiment of the cartridge platform 141 shown in FIG. 8 also includes a set of valve actuation slots 145, oriented perpendicular to the parallel cartridge loading guiderails 142 and configured to provide access to a valve actuation subsystem 170, and a magnet receiving slot 144 located among the set of valve actuation slots 145. Preferably, the magnet receiving slot 144 and the set of valve actuation slots 145 substantially span a long dimension of the cartridge platform 141, as shown in FIG. 8, and are configured to correspond to locations on a microfluidic cartridge 210 requiring a magnetic field and/or valving to enable processing of a biological sample and nucleic acid detection once the microfluidic cartridge 210 has been aligned within the molecular diagnostic module 130. Thus, alternative configurations of the magnet receiving slot 144 and the set of valve actuation slots 145 may accommodate other cartridges with alternative regions requiring magnetic fields and/or valving to enable other protocols. In one alternative embodiment, the magnet receiving slot 144 and

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the set of valve actuation slots may comprise one continuous void of the cartridge platform 141, such that the cartridge platform 141 supports a microfluidic cartridge 210 along the periphery of the microfluidic cartridge 210, but forms a continuous void under a majority of the footprint of the 5 microfluidic cartridge 210.

The linear actuator 146 functions to linearly displace a microfluidic cartridge 210 resting on the cartridge platform 141, in order to compress the microfluidic cartridge 210 and position the microfluidic cartridge 210 between a cartridge heater 153 and an optical subsystem 180 on one side of the microfluidic cartridge 210, and a magnet 160 and detection chamber heaters 157 on another side of the microfluidic cartridge 210. The linear actuator 146 also functions to provide a sufficient counterforce to the valve actuation 15 subsystem 170 such that a microfluidic cartridge 210 within the molecular diagnostic module 130 remains properly situation upon manipulation by the valve actuation subsystem 170. The linear actuator 146 further functions to move a nozzle 149 coupled to the liquid handling system 250, in 20 order to couple the liquid handling system 250 to a fluid port 222 of the microfluidic cartridge 210. In the orientation of the molecular diagnostic module 130 shown in FIGS. 7B and 7B, the linear actuator 146 is preferably coupled to a portion of the heating and cooling subsystem 150 a portion 25 of the optical subsystem 180, and the nozzle 149, and vertically displaces the cartridge heater 153, the optical subsystem 180, and the nozzle 149 to position the cartridge heater 153, 180 and the nozzle 149 over the microfluidic cartridge 210. The vertical displacement also allows the 30 microfluidic cartridge 210 to receive a magnet 160, which provides a magnetic field to facilitate a subset of a molecular diagnostic protocol, and detection chamber heaters 157, which allows amplification of nucleic acids for molecular diagnostic protocols requiring heating and cooling of the 35 nucleic acid (e.g. PCR). Preferably, the linear actuator 146 is a scissor jack actuator configured to apply substantially uniform pressure over all occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and to operate in at least two configurations. In 40 a retracted configuration 146a, as shown in FIG. 9A, the scissor jack actuator has not linearly displaced the cartridge platform 141, and in an extended configuration 146b, as shown in FIG. 9B, the scissor jack actuator has linearly displaced the microfluidic cartridge 210 to position the 45 microfluidic cartridge 210 between the subsystems 153, and 180, and the magnet 160 and detection chamber heaters 157. Additionally, the extended configuration 146b of the scissor jack actuator is configured to couple the nozzle 149 to a fluid port 222 of the microfluidic cartridge 210, such that the 50 liquid handling system 250 can deliver solutions and gases for processing of biological samples. The linear actuator 146 may alternatively be any appropriate linear actuator, such as a hydraulic, pneumatic, or motor-driven linear actuator, configured to linearly displace a microfluidic cartridge 55 within the molecular diagnostic module 130.

As shown in FIGS. 7B, 7C, and 8, a set of springs 148 is coupled to the cartridge platform 141 and functions to provide a counteracting force against the linear actuator 146 as the linear actuator 146 displaces a microfluidic cartridge 60 210 resting on the cartridge platform 141. The set of springs 148 thus allows the cartridge platform 141 to return to a position that allows the microfluidic cartridge 210 to be loaded and unloaded from the molecular diagnostic module 130 when the linear actuator 146 is in a retracted configuration 146b, as shown in FIG. 7B. Preferably, in the orientation shown in FIG. 7B, the set of springs 148 is located at

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peripheral regions of the bottom side of the cartridge platform 141, such that the set of springs 148 does not interfere with the magnet or the valve actuation subsystem 170. Alternatively, the set of springs 148 may be located at any appropriate position to provide a counteracting force against the linear actuator 146. In a specific example shown in FIG. 6A, the set of springs 148 comprises four springs located near corners of the bottom side of the cartridge platform 141, but in other variations, the set of springs 148 may comprise any appropriate number of springs. Each spring of the set of springs 148 is also preferably housed within a guide to prevent deviations from linear vertical motions (in the orientation shown in FIG. 7B); however, each spring in the set of springs 148 may alternatively not be housed within a guide. In an alternative embodiment of the molecular diagnostic module 130, the set of springs 148 may altogether be replaced by a second linear actuator configured to linearly displace a microfluidic cartridge 210, resting on the cartridge platform 141, in a direction opposite to the displacements enforced by the linear actuator 146.

Similarly, the nozzle 149, the heating and cooling subsystem 150, the cartridge heater 153, and the magnet 160 are preferably coupled to springs, such that springs are positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. Alternatively an elastomeric material is preferably positioned between elements 149, 150, 153, and 160, and substrates that elements 149, 150, 153, and 160 are mounted to. The springs and/or elastomeric material function to provide proper functioning and alignment of subsystems of the molecular diagnostic module 130 as the linear actuator 146 is extended or retracted, contributing to reliability and a reduction in stack up tolerance risk. The springs and/or elastomeric material further function to allow more pressure to be applied to occlusion positions of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, and an appropriate pressure to be applied to elements 149, 150, 153 and 160 of the molecular diagnostic module 130. Thus, proper contact is maintained between elements 149, 150, 153, and 160, and a microfluidic cartridge 210 being manipulated by the molecular diagnostic module. These elements are described in further detail below. 1.2.2 Molecular Diagnostic Module—Heating/Cooling Subsystem and Magnet

The heating and cooling subsystem 150 of the molecular diagnostic module 130 comprises a cartridge heater 153, a fan 155, and a set of detection chamber heaters 157 and functions to controllably heat portions of a microfluidic cartridge 210 for processing of a biological sample containing nucleic acids according to a molecular diagnostic protocol. In the orientation of an embodiment of the molecular diagnostic module 130 shown in FIGS. 7A-7C, the cartridge heater 153 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140 and configured to span a central region of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130, the fan 155 is located at a back wall of the cartridge receiving module 140, and the set of detection chamber heaters 157 is located inferior to a set of detection chambers 213 of the microfluidic cartridge 210. In alternative embodiments of the molecular diagnostic module 130, the heating and cooling subsystem 150 may have any appropriate alternative configuration that provides controlled heating and cooling to a microfluidic cartridge within the molecular diagnostic module 130.

The cartridge heater 153 functions to transfer heat to a heating region 224 of a microfluidic cartridge 210, for inducing a pH shift to release bound nucleic acids from

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magnetic beads within the heating region 224. The cartridge heater 153 is preferably a plate-shaped heater configured to transfer heat to the microfluidic cartridge 210 only from one side of the cartridge heater 153, such that heat flows through one face of the plate-shaped heater to the microfluidic cartridge 210. In a specific example, the cartridge heater 153 is a silicon wafer etched to be conductive and form a resistance heater. In the preferred variation, the cartridge heater 153 is either flip-chip bonded (i.e., soldered to back side of a circuit board), or wire bonded to a circuit board, and then coupled using linear bearings and springs to a plate coupled to the linear actuator 146. The preferred variation allows independent control of 12 independent channels, corresponding to 12 different pathways for sample processing. In another variation, heating through one face is accom- 15 plished using a plate-shaped resistance heater that has one exposed face and thermal insulation covering all other faces, and in yet another variation heating through one face is accomplished using a Peltier heater. In a variation of the cartridge heater 153 using a Peltier heater, the cartridge 20 heater 153 comprises a thermoelectric material, and produces different temperatures on opposite faces of the cartridge heater 153 in response to a voltage difference placed across the thermoelectric material. Thus, when a current flows through the Peltier heater, one face of the Peltier heater 25 lowers in temperature, and another face of the Peltier heater increases in temperature. Alternative variations of the cartridge heater 153 can be used to appropriately transfer heat to a heating region 224 of the microfluidic cartridge 210.

Preferably, the cartridge heater 153 is configured to linearly translate with the linear actuator 146 of the cartridge receiving module 140, in order to align with a heating region 224 spanning a central portion of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In one variation, the cartridge heater 153 is preferably fixed 35 relative to the linear actuator 146 such that (in the orientation shown in FIGS. 7B-7C), the cartridge heater 153 can only move vertically with the linear actuator. In an alternative variation, the cartridge heater 153 may additionally be configured to translate laterally with a horizontal plane (in 40 the orientation shown in FIGS. 7B-7C), such that the cartridge heater 153 can translate in at least two perpendicular coordinate planes. In this alternative variation, the cartridge heater 153 can be configured to sweep across a surface of a microfluidic cartridge 210 aligned within the molecular 45 diagnostic module 130, or to translate in response to motion of the microfluidic cartridge 210, such that the position of the cartridge heater 153 relative to a heating region 224 of the microfluidic cartridge 210 is always fixed.

The fan 155 functions to modulate heat control within the 50 molecular diagnostic module 130, by enabling heat transfer from warm objects within the molecular diagnostic module 130 to cooler air external to the molecular diagnostic module 130. In the orientation shown in FIG. 6A, the fan 155 is preferably located at a back face of the molecular diagnostic 55 module 130, such heat within the molecular diagnostic module 130 is transferred out of the back face of the molecular diagnostic module 130 to cooler air external to the molecular diagnostic module. In a specific embodiment, the molecular diagnostic module 130 comprises four fans 155 60 located at the back face of the molecular diagnostic module 130; however, in alternative embodiments the molecular diagnostic module 130 may comprise any appropriate number of fans located at any appropriate position of the molecular diagnostic module 130. In one variation, the fan 65 155 may be passive and driven solely by convection currents resulting from motion of hot air within the molecular

diagnostic module to cooler air outside of the molecular diagnostic module; however, in alternative variations, the fan 155 may be motor-driven and configured to actively cool internal components of the molecular diagnostic module 130 if molecular diagnostic module elements exceed a certain threshold temperature.

The set of detection chamber heaters 157 functions to individually heat detection chambers of a set of detection chambers 213 within a microfluidic cartridge 210. Each detection chamber heater in the set of detection chamber heaters 157 is preferably configured to heat one side of one detection chamber in the set of detection chambers 213, and is preferably located such that the extended configuration **146**b of the linear actuator **146** of the cartridge receiving module 140 puts a detection chamber in proximity to a detection chamber heater. As mentioned above, the set of detection chamber heaters 157 is preferably coupled to springs or an elastomeric layer to ensure direct contact between the set of detection chamber heaters and a set of detection chambers, without compressively damaging the set of detection chamber heater 157. Preferably, each detection chamber heater is configured to contact a surface of a detection chamber in the extended configuration 146b of the linear actuator 146; however, each detection chamber heater may be further configured to couple to a detection chamber in the extended configuration 146b of the linear actuator **146**. In a first variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a flexible printed circuit board, with a set of springs coupled to an opposite surface of the flexible printed circuit board, such that each spring in the set of springs aligns with a detection chamber heater. In the first variation, contact between each detection chamber heater and a detection chamber is thus maintained by a biasing force provided by an individual spring through the flexible printed circuit board. In a second variation, the set of detection chamber heaters 157 comprises silicon chip heaters flip chipped to one surface of a rigid printed circuit board, with a set of springs coupled to an opposite surface of the rigid printed circuit board. In the second variation, the set of springs thus function to collectively transfer a force through the rigid printed circuit board to maintain contact between the set of detection chamber heaters and a set of detection chambers. Preferably, each detection chamber heater in the set of detection chamber heaters 157 is configured to contact and heat a bottom surface of a detection chamber (in the orientation shown in FIG. 7B); however, each detection chamber heater may alternatively be configured to contact and heat both a top and a bottom surface of a detection chamber. Additionally, each detection chamber heater preferably corresponds to a specific detection chamber of the set of detection chambers 213 and functions to individually heat the specific detection chamber; however, alternatively, each detection chamber heater may be configured to heat multiple detection chambers in the set of detection chambers 213. Preferably, all detection chamber heaters in the set of detection chamber heaters 157 are identical; however, the set of detection chamber heaters 157 may alternatively not comprise identical detection chamber heaters.

In one variation, each detection chamber heater in the set of detection chamber heaters 157 comprises a donut-shaped heater, configured to encircle a surface of a detection chamber. The donut-shaped heater may further include a conducting mesh configured to allow detection through the heater while still allowing efficient heat transfer to the detection chamber. In an alternative variation, each detection chamber heater in the set of detection chamber heaters 157 may

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include a plate-shaped Peltier heater, similar to Peltier cartridge heater 153 described above. In this alternative variation, each detection chamber heater is thus configured to heat one side of a detection chamber through one face of the detection chamber heater. In one specific example, the 5 molecular diagnostic module 130 comprises 12 diced silicon wafers with conductive channels flip chipped to 12 detection chambers, providing resistive heating to each of the 12 detection chambers. In another specific example, the molecular diagnostic module 130 comprises a 12 Peltier detection chamber heaters configured to heat 12 detection chambers of a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. In other alternative variations, each detection chamber heater may comprise any appropriate heater configured to individually heat a detec- 15 tion chamber.

The magnet 160 of the molecular diagnostic module 130 functions to provide a magnetic field for isolation and extraction of nucleic acids bound to magnetic beads within a microfluidic cartridge 210, aligned within the molecular 20 diagnostic module 130. Preferably, the magnet 160 is fixed within the molecular diagnostic module 130, such that the extended configuration 146b of the linear actuator 146 allows the magnet 160 to pass through the magnet receiving slot 144 of the cartridge receiving module 140 and into a 25 magnet housing region 218 of the microfluidic cartridge 210. In an example, as shown in FIGS. 7A-7C, the magnet 160 is a rectangular prism-shaped magnet 160 fixed under the cartridge platform 141, and configured to pass through the cartridge platform 141, into a magnet housing region 218 30 located under the heating region 224 of the microfluidic cartridge 210. Preferably, the magnet 160 is one of two or three magnets lined up in parallel, such that each of the fluidic pathways of a microfluidic cartridge housing the magnets is exposed to two or three times as much magnetic 35 flux, and two to threes times as many opportunities to capture magnetic beads. Alternatively, the magnet 160 is a single magnet configured to expose a set of fluidic pathways to a magnetic field. Preferably, the magnet 160 or group of multiple magnets is coupled to a magnet holder within the 40 molecular diagnostic module 130. Additionally, the magnet holder is preferably composed of an insulating material, such that the magnet holder does not interfere with proper functioning of the cartridge heater 153. Alternatively, the magnet holder may not be composed of an insulating 45 material.

In one variation, the magnet 160 or group of multiple magnets comprises a permanent magnet, composed of a magnetized material (e.g., a ferromagnet) providing a substantially fixed magnetic field. In an alternative variation, the 50 magnet 160 or group of multiple magnets comprises an electromagnet configured to provide a modifiable magnetic field, such that the intensity of the magnetic field can be adjusted, the polarity of the magnetic field can be reversed, and the magnetic field can be substantially removed upon 55 removal of a current flowing within the electromagnet. Preferably, the magnet 160 or group of magnets is also fixed relative to the molecular diagnostic module 130; however, the magnet 160 or group of magnets may alternatively be configured to translate vertically (in the orientation shown in 60 FIG. 7B), such that the magnet 160 or group of magnets can extend into and retract from the magnet receiving slot 144 of the cartridge platform 141 and the magnet housing region 218 of the microfluidic cartridge 210. Additionally, the magnet 160 or group of magnets preferably rides on linear 65 bearings and springs (or an elastomeric material) to ensure proper contact with a microfluidic cartridge in an extended

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configuration **146***b* of the linear actuator **146**, in a manner that allows most of force from the linear actuator **146** to translate to full occlusion of a subset of the set of occlusion positions (i.e., without leakage).

Alternative configurations and/or compositions of the magnet 160 may also be appropriate in facilitating isolation and extraction of nucleic acids bound to magnetic beads within the microfluidic cartridge 210.

1.2.3 Molecular Diagnostic Module—Valve Actuation Subsystem

As shown in FIGS. 10A-11C, the valve actuation subsystem 170 of the molecular diagnostic module 130 comprises a set of pins 172 configured to translate linearly within a pin housing 175, by sliding a cam card 177 laterally over the pins 172. The valve actuation subsystem 170 functions to provide a biasing force to deform an object in contact with the set of pins 172. In a configuration wherein a microfluidic cartridge 210 is aligned within the molecular diagnostic module 130, the valve actuation subsystem 170 thus functions to occlude a fluidic pathway 220 of the microfluidic cartridge 210 at a set of occlusion positions 226, to control flow of a biological sample containing nucleic acids, reagents and/or air through the microfluidic cartridge 210. In an embodiment of the molecular diagnostic module shown in FIGS. 7D-7E, the set of pins 172 and the pin housing are located directly under the microfluidic cartridge 210, such that the set of pins can access the microfluidic cartridge 210 through the valve actuation accommodating slots 145 of the cartridge platform 141. The cam card 177 in the embodiment is positioned under the set of pins and is coupled to a linear cam card actuator 178 configured to laterally displace the cam card 177 to vertically displace pins of the set of pins 172. Preferably, as shown in FIG. 11A, the cam card 177 rests on a low friction surface configured to facilitate lateral displacement of the cam card 177; however, the cam card 177 may alternatively rest on a bed of ball bearings to facilitate lateral displacement of the cam card 177, or may rest on any feature that allows the cam card 177 to be laterally displaced by the linear cam card actuator 178.

The cam card 177, as shown in FIGS. 7D and 11A, includes a set of hills 176 and valleys 179, and functions to transform linear motion in one plane to vertical motion in another plane. In one variation, the cam card 177 is coupled to a linear actuator and contacts the ends of pins in a set of pins 172, such that when a hill 176 of the cam card 177 passes under a pin, the pin is in a raised configuration 177a, and when a valley 179 of the cam card 177 passes under a pin, the pin is in a lowered configuration 177b. The hills 176 and valleys 179 of the cam card 177 are preferably in a set configuration, as shown in FIG. 11B, such that lateral motion of the cam card 177 to a set position raises a fixed subset of the set of pins 172. In this manner, lateral movement of the cam card 177 to different positions of a set of positions consistently raises different subsets of the set of pins 172 to occlude different portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172. Thus, portions of a fluidic pathway 220 may be selectively occluded and opened to facilitate processing of a biological sample according to any appropriate tissue, cellular, or molecular diagnostic assay protocol. In one variation, the cam card is configured to be laterally displaced in two coordinate directions within a plane (e.g., by x-y linear actuators), and in another variation, the cam card is configured to be laterally displaced in only one coordinate direction within a plane (e.g., by a single linear actuator). In a specific example, the hills 176 of the cam card 177 are raised 1 mm above the valleys 179 of the cam card 177, the hills

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176 and valleys 179 each have a 2 mm wide plateau region, and a hill 176 region slopes down to a valley region 179 at a fixed angle over a 2 mm length. In the specific example, the cam card 177 is driven by a Firgelli linear actuator. Alternative variations may include any appropriate configurations and geometries of a cam card with hills 176 and valleys 179, driven by any appropriate actuator.

In alternative embodiments of the valve actuation subsystem 170, the cam card 177 may be a cam card wheel comprising a set of hills 176 and valleys 179 on a cylindrical surface, and configured to convert rotary motion to linear (i.e., vertical) motion of the set of pins 172. The cam card wheel may be configured to contact ends of pins in the set of pins 172, and may be coupled to a motor shaft and driven by a motor. In other alternative embodiments of the valve actuation subsystem 170, the cam card 177 may altogether be replaced by a set of cams, each configured to individually rotate about an axis. In these alternative embodiments, rotating subsets of the set of cams raises corresponding subsets of the set of pins, and occludes specific portions of a fluidic pathway 220 of a microfluidic cartridge 210 in contact with the set of pins 172.

The set of pins 172 functions to selectively occlude portions of a fluidic pathway 220 of a microfluidic cartridge 25 210 at least at subsets of a set of occlusion positions 226. The pins of the set of pins 172 are preferably cylindrical and, in the orientation shown in FIG. 11A, configured to slide over a cam card 177 and within a pin housing 175. Each pin in the set of pins 172 preferably also includes a first spring 30 173 that functions to provide a counteracting force to restore a pin to a lowered configuration 177b; however, each pin in the set of pins 172 may alternative not include a first spring 173, and rely solely on gravity to return to a lowered configuration 177b. Preferably, as shown in FIG. 11C, each 35 pin is also composed of two parts separated by a second spring, which functions to allow sufficient force to fully occlude a microfluidic channel but prevents forces from being generated that could damage the pin, microfluidic cartridge and/or cam card. Each pin also preferably com- 40 prises a first region 171 configured to slide within the pin housing 175, and a second region 174 configured to exit the pin housing 175. The second region 174 is preferably of a smaller dimension than the first region 171, such that each pin is constrained by the pin housing 175 to be raised by a 45 limited amount. Alternatively, the first region 171 and the second region 174 may have any appropriate configuration to facilitate raising and lowering of a pin by a fixed amount. In a specific example, the valve actuation subsystem 170 comprises 12 sets of pins 172 configured to selectively 50 occlude 12 fluidic pathways 212 of a microfluidic cartridge 210 aligned within the molecular diagnostic module; however, other embodiments may comprise any appropriate number of sets of pins 172.

In the orientation shown in FIG. 11A, each pin in the set 55 of pins 172 preferably has a circular cross section and round ends, configured to facilitate sliding within a pin housing 175, sliding over a cam card 177 surface, and occlusion of a fluidic pathway 220. Alternatively, each pin may comprise any appropriate cross-sectional geometry (e.g., rectangular) 60 and/or end shape (e.g., flat or pointed) to facilitate occlusion of a fluidic pathway 220. Preferably, the surface of each pin in the set of pins 172 is composed of a low-friction material to facilitate sliding motions (i.e., over a cam card 177 or within a pin housing 175); however, each pin may alternatively be coated with a lubricant configured to facilitate sliding motions.

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The pin housing 175 functions to constrain and guide the motion of each pin in the set of pins 172, as the cam card 177 slides under the set of pins 172. Preferably, the pin housing 175 comprises a set of pin housing channels 169 configured to surround at least one pin in the set of pins 172. In one variation, each pin in the set of pins 172 is surrounded by an individual channel of the set of pin housing channels 169; however, in another variation a channel of the set of pin housing channels 169 may be configured to surround multiple pins in the set of pins 172. In an example shown in FIGS. 7D-7E and 11A, the pin housing is located under the cartridge platform 141, such that the set of pin housing channels 169 is aligned with the set of valve actuation accommodating slots 145, to provide access, by the set of pins 172, to a microfluidic cartridge 210 aligned on the cartridge platform 141. In the example, the pin housing 175 thus constrains the set of pins 172, such that each pin can only move linearly in a vertical direction. Each pin housing channel preferably has a constricted region 168 configured to limit the motion of a pin within a pin channel; however, each pin housing channel may alternatively not include a constricted region. Preferably, surfaces of the pin housing 175 contacting the set of pins 172 are composed of a low friction material to facilitate sliding of a pin within a pin housing channel; however, surfaces of the pin housing 175 contacting the set of pins 172 may alternatively be coated with a lubricant configured to facilitate sliding motions. Other variations of the pin housing 175 and the set of pins 172 may include no additional provisions to facilitate sliding of a pin within a pin housing channel.

1.2.4 Molecular Diagnostic Module—Optical Subsystem

As shown in FIGS. 12A-12D, the optical subsystem 180 of the molecular diagnostic module 130 comprises a set of light emitting diodes (LEDs) 181, a set of excitation filters 182 configured to transmit light from the set of LEDs 181, a set of dichroic mirrors 183 configured to reflect light from the set of excitation filters 182 toward a set of apertures 185 configured to transmit light toward a set of nucleic acid samples, a set of emission filters 186 configured to receive and transmit light emitted by the set of nucleic acid samples, and a set of photodetectors 187 configured to facilitate analysis of light received through the set of emission filters **186**. The optical subsystem **180** may further comprise a set of lenses 184 configured to focus light onto the set of nucleic acid samples. The optical subsystem 180 thus functions to transmit light at excitation wavelengths toward a set of nucleic acid samples and to receive light at emission wavelengths from a set of nucleic acid samples. Preferably, the optical subsystem 180 is coupled to an optical subsystem actuator 188 configured to laterally displace and align the optical subsystem 180 relative to the set of nucleic acid samples, and is further coupled to a linear actuator 146 of the cartridge receiving module 140 to position the optical subsystem 180 closer to the set of nucleic acid samples. Alternatively, the optical subsystem 180 may not be coupled to a linear actuator 146 of the cartridge receiving module 140, and may only be configured to translate laterally in one direction. In a specific example, the optical subsystem 180 comprises a set of 12 apertures, a set of 12 lenses, a set of 12 dichroic mirrors, a set of 12 excitation filters, a set of 12 LEDs, a set of 12 emission filters, and a set of 12 photodetectors. In the specific example, as shown in FIG. 7A-7E, the optical subsystem 180 is located within the molecular diagnostic module 130 and coupled to the linear actuator 146 of the cartridge receiving module 140, such that, in the extended configuration 146b of the linear actuator 146, the optical subsystem 180 can be positioned closer to a micro-

fluidic cartridge 210 aligned within the molecular diagnostic module. Conversely in the specific example, the optical subsystem 180 is positioned away from the microfluidic

cartridge 210 in the retracted configuration 146a of the linear actuator 146. In the specific example, the optical 5 subsystem 180 is further coupled to an optical subsystem actuator 188 configured to laterally displace the optical subsystem 180 relative to the microfluidic cartridge 210, such that the optical subsystem 180 can be aligned with a set of detection chambers 213 of the microfluidic cartridge 210. 10

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Preferably, the set of LEDs 181 are not all identical but rather chosen to efficiently produce a certain band of wavelengths of light, such that light from the set of LEDs 181 can be filtered to appropriate narrow wavelengths for analysis of nucleic acid samples. Alternatively, all LEDs in the set of 15 187. LEDs 181 may be identical, and produce white light comprising all wavelengths of visible light that is filtered to produce the desired wavelength, in which case the LEDs may be stationary. Preferably, the set of LEDs 181 includes phosphor-based LEDs, but the set of LEDs 181 may alter- 20 natively include any LEDs configured to provide light of the desired range of wavelengths. The LEDs of the set of LEDs 181 are preferably configured to emit light of wavelengths corresponding to at least one of the set of excitation filters 182, the set of dichroic mirrors 183, and the set of emission 25 filters 186.

The set of excitation filters 182 is configured to align with the set of LEDs 181 in the optical subsystem 180, and functions to transmit light at excitation wavelengths toward the set of dichroic mirrors 183 of the optical subsystem 180. 30 Preferably, the set of excitation filters 182 are not identical excitation filters, but rather chosen to transmit the different desired ranges of excitation wavelengths. Alternatively, all excitation filters of the set of excitation filters 182 are identical, and configured to transmit light having a fixed 35 range of excitation wavelengths. In one variation, the set of excitation filters 182 includes band pass filters, configured to transmit light between two bounding wavelengths, in another variation, the set of excitation filters 182 includes wavelength, and in yet another variation, the set of excitation filters 182 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of excitation filters 182 is interchangeable, such that individual excitation filters may be interchanged to provide 45 different excitation wavelengths of light; however, the set of excitation filters 182 may alternatively be fixed, such that the optical subsystem 180 is only configured to transmit a fixed range of excitation wavelengths.

The set of dichroic mirrors 183 is configured to align with 50 the set of excitation filters 182, and functions to receive and reflect light from the set of excitation filters 182 toward the detection chamber, such that light having a range of excitation wavelengths may be focused, through a set of apertures, onto a set of nucleic acid samples. The set of dichroic 55 mirrors 183 also functions to receive and transmit light from a set of emission filters 186 toward a set of photodetectors **187**, which is described in more detail below. All dichroic mirrors in the set of dichroic mirrors 183 are preferably identical in orientation relative to the set of excitation filters 60 182 and the set of emission filters 186, and configured to reflect and transmit the appropriate wavelengths of light for the given LED. Alternatively, the set of dichroic mirrors 183 may include identical dichroic mirrors, with regard to orientation, light transmission, and light reflection. In a specific 65 example, in the orientation shown in FIG. 12A, the set of excitation filters 182 is oriented perpendicular to the set of

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emission filters 186, with the set of dichroic mirrors 183 bisecting an angle between two planes formed by the faces of the set of excitation filters 182 and the set of emission filters 186. In the specific example, light from the set of excitation filters is thus substantially reflected at a 90° angle toward the set of apertures 185, and light from the set of emission filters 186 passes in a substantially straight direction through the set of dichroic mirrors 183 toward the set of photodetectors 187. Other variations of the set of dichroic mirrors 183 may include any configuration of dichroic mirrors, excitation filters, and/or emission filters that enable transmission of light of excitation wavelengths toward a set of nucleic acid samples, and transmission of light from the set of nucleic acid samples toward a set of photodetectors

In one embodiment, the optical subsystem may further include a set of lenses 184 configured to align with the set of dichroic mirrors 183, which functions to focus light, from the set of excitation filters 182 and reflected off of the set of dichroic mirrors 183, onto a set of nucleic acid samples configured to emit light in response to the light from the set of excitation filters 182. All lenses in the set of lenses 184 are preferably identical in orientation relative to the set of dichroic mirrors and in dimension; however, the set of lenses 184 may alternatively comprise non-identical lenses, such that light passing through different lenses of the set of lenses **184** is focused differently on different nucleic acid samples. In a specific example, in the orientation shown in FIG. 12A, the faces of the set of lenses 184 are oriented perpendicular to the faces of the set of excitation filters 182, to account for light reflection by the set of dichroic mirrors 183 at a 90° angle. In the specific example, the set of lenses also includes identical 1/4" high numerical aperture lenses. In other variations, the set of lenses 184 may be oriented in any appropriate configuration for focusing light from the set of dichroic mirrors 183 onto a set of nucleic acid samples, and may include lenses of any appropriate specification (i.e., numerical aperture).

The set of apertures 185 is located on an aperture substrate short pass filters configured to transmit light below a certain 40 189 and configured to align with the set of lenses 184, and functions to allow focused light from the set of lenses 184 to pass through to the set of nucleic acid samples. The aperture substrate 189 is preferably coupled to the linear actuator 146 of the cartridge receiving module 140, which allows the optical subsystem 180 to linearly translate and be positioned near and away from a microfluidic cartridge 210 aligned within the molecular diagnostic module 130. Alternatively, the aperture substrate 189 may not be coupled to the linear actuator 146 of the cartridge receiving module 140. Preferably, all apertures 185 in the set of apertures 185 are identical, and configured to allow identical light profiles to be focused, through the set of lenses 184, onto a set of nucleic acid samples. Alternatively, the set of apertures 185 may not include identical apertures. In one variation, each aperture in the set of apertures 185 may be individually adjustable, in order to provide individually modifiable aperture dimensions (e.g., width, length, or diameter) to affect light exposure. In an alternative variation, each aperture in the set of apertures 185 is fixed. Other variations may include interchangeable aperture substrates 189, such that features of the set of apertures (e.g., aperture dimensions, number of apertures) may be adjusted by interchanging aperture substrates 189.

The set of emission filters 186 is configured to align with the set of dichroic mirrors, and functions to transmit emission wavelengths of light from the set of nucleic acid samples, and to filter out excitation wavelengths of light.

Preferably, each emission filter of the set of emission filters 186 are configured to transmit light having a fixed range of emission wavelengths, while blocking light of excitation wavelengths. Alternatively, the set of emission filters 186 may comprise identical emission filters, such that individual 5 emission filters of the set of emission filters 186 are configured to transmit the same ranges of emission wavelengths. In one variation, the set of emission filters 186 includes band pass filters, configured to transmit light the set of emission filters 186 includes short pass filters configured to transmit light below a certain wavelength, and in yet another variation, the set of emission filters 186 includes long pass filters configured to transmit light above a certain wavelength. Preferably, the set of emission filters 15 186 is interchangeable, such that individual emission filters may be interchanged to transmit and/or block different wavelengths of light; however, the set of emission filters 186 may alternatively be fixed, such that the optical subsystem

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The set of photodetectors 187 is configured to align with the set of emission filters 186, and functions to receive light from the set emission filters to facilitate analysis of the set of nucleic acid samples. All photodetectors in the set of 25 photodetectors 187 are preferably identical; however, the set of photodetectors 187 may alternatively include non-identical photodetectors. Preferably, the set of photodetectors 187 includes photodiodes comprising a photoelectric material configured to convert electromagnetic energy into elec- 30 trical signals; however, the set of photodetectors 187 may alternatively comprise any appropriate photodetectors for facilitating analysis of biological samples, as is known by those skilled in the art.

**180** is only configured to transmit a fixed range of emission 20

The optical subsystem actuator 188 is coupled to the 35 optical subsystem 180, and functions to laterally translate the optical subsystem 180 relative to a set of nucleic acid samples being analyzed. Preferably, the optical subsystem actuator 188 is a linear actuator configured to translate the optical subsystem 180 in one dimension; however, the 40 optical subsystem actuator 188 may alternatively be an actuator configured to translate the optical subsystem 180 in more than one dimension. In a specific example, as shown in FIGS. 7A-7D and 12D, the optical subsystem actuator 188 is configured to translate the optical subsystem 180 45 laterally in a horizontal plane, to align the optical subsystem 180 with a set of detection chambers 213 of a microfluidic cartridge 210 within the molecular diagnostic module 130. In another example, the optical subsystem may be configured as a disc revolving around an axis with the LEDs and 50 photodetectors stationary and the disc containing the filters. In other variations, the optical subsystem actuator 188 may be configured in any appropriate manner to facilitate alignment of the optical subsystem 180 relative to a set of nucleic acid samples being analyzed.

1.2.5 Molecular Diagnostic Module—Alternative Embodiments and Variations

As described above, alternative embodiments of the molecular diagnostic module 130 and alternative variations of subsystems and elements of the molecular diagnostic 60 module 130 may be configured to process a biological sample containing nucleic acids, isolate nucleic acids from the biological sample, and detect nucleic acids. An example of an alternative embodiment of a molecular diagnostic module 130, as shown in FIG. 13, includes a cartridge 65 receiving module 140', a heating and cooling subsystem 150', a magnet 160', a valve actuation subsystem 170', and

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an optical subsystem 180', and functions to manipulate an alternative microfluidic cartridge 210' for processing of biological samples containing nucleic acids. Other alternative embodiments of the molecular diagnostic module 130" may be configured to receive alternative microfluidic cartridges 210", for processing of biological samples containing nucleic acids.

1.3 System—Assay Strip

As shown in FIGS. 18A and 18B, the assay strip 190 between two bounding wavelengths, in another variation, 10 comprises an assay strip substrate 191 comprising a set of wells 192, and typically a puncturable foil seal 195, and functions to facilitate combination of a set of nucleic acid samples with a set of molecular diagnostic reagents for amplification and/or detection of a nucleic acid sequence or sequences. Preferably, the entire assay strip 190 is configured to be a consumable (i.e., disposable), such that the assay strip 190 can be used during multiple runs of the system 100, then the assay strip 190 is disposed of once all of the wells 192, containing unitized reagents for a single test or group of tests, is exhausted. Alternatively, at least a portion of the assay strip 190 is configured to be reusable, such that wells may be reloaded with reagents and reused with the system 100. In one variation of the assay strip 190, the assay strip substrate 191 is reusable, while the puncturable foil seal 195 is disposable and replaced after each run of the system 100. In another variation, the reusable assay strip substrate 191 does not require a puncturable foil seal 195, such that reagents specific to a certain nucleic acid sequences may be deposited into open wells of the assay strip substrate 191 by a user.

> The assay strip substrate 191 is configured such that the assay strip 190 is capable of resting on a flat surface, and functions to define the set of wells 192 and to couple to the puncturable foil seal 195. The assay strip substrate 191 is preferably configured to be received by a corresponding assay strip holder 230 configured to hold multiple assay strips 190, but may alternatively not be configured to couple to an assay strip holder 230. The assay strip substrate 191 is preferably composed of a PCR-compatible polymer, such as polypropylene, that can be heat processed to couple to the puncturable foil seal 115, but can alternatively be composed of any appropriate material that can contain a fluid and be bonded to the puncturable foil seal 115.

The set of wells 192 of the assay strip substrate 191 function to receive at least one nucleic acid sample, and to facilitate combination of the nucleic acid sample with at least one of a set of molecular diagnostic reagents. The molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to perform alternative molecular diagnostic protocols. Preferably, the wells 193 of the assay strip substrate 191 are each configured to accommodate not only a nucleic acid sample, but also to facilitate mixing of the nucleic acid sample with at least one of a set of molecular diagnostic reagents (e.g., using a pipettor or other apparatus). Additionally, the molecular diagnostic reagents of the set of molecular diagnostic reagents preferably comprises probes and primers to detect the sample process controls provided by the capture plate, in order to verify process fidelity and assay accuracy. Preferably, the wells 193 are deep enough to facilitate

mixing without splashing, and evenly spaced to facilitate aspiration, delivery, and/or mixing of multiple biological samples (e.g., with a multi-tip pipettor). Alternatively, the wells are wide and shallow to facilitate drying of reagents in the wells to increase shelf life and larger devices for mixing 5 the nucleic acids with molecular diagnostic reagents. Each well 193 of the set of wells 192 also preferably has a rounded bottom region, as shown in FIG. 18A, to facilitate complete aspiration of a fluid from a well 193; however, each well 193 may alternatively not have a rounded bottom region. Additionally, the set of wells 192 is preferably arranged in staggered rows, which functions to facilitate access to individual wells 193 of the set of wells, to reduce one dimension

of the assay strip 190, and also to prevent cross-contamina-

tion of fluids within the wells due to dripping. Alternatively, 15

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the set of wells 192 may not be arranged in staggered rows. The puncturable foil seal 195 functions to protect the molecular diagnostic reagents stored in wells 112 from degradation, isolate each well 193 of the set of wells 192, prevent contamination of the contents of each of the set of 20 wells 192, and provide information identifying the assay strip 190. The puncturable foil seal 195 preferably seals each well 193 of the assay strip 190, and is configured to be punctured by an external element (e.g., by a pipette tip), such that each well is sealed prior to being punctured. In one 25 variation, the puncturable foil seal 195 also forms a seal around an element that punctures it, and in another variation, the puncturable foil seal 195 does not form a seal around an element that punctures it, in order to prevent airlock. The puncturable foil seal 195 is also preferably labeled with 30 identifying information including at least one of manufacturer information, assay strip contents, the lot of the contents, an expiry date, and a unique electronic tag (e.g., barcode or QR code) providing more information. Preferably, the puncturable foil seal 195 does not extend beyond 35 the footprint of the assay strip 190, but alternatively, the puncturable foil seal 195 may be any appropriate size and/or include protruding features (e.g., tabs) that facilitate handling of the assay strip.

In one variation, the assay strip 190 may be prepackaged 40 with a set of molecular diagnostic reagents, such that each well 193 in the set of wells 192 is prepackaged with a quantity of molecular diagnostic reagents. The set of wells 192 may then be sealed by the puncturable foil seal 195, which is configured to be punctured by an external element 45 that delivers volumes of nucleic acid samples to be combined with the set of molecular diagnostic reagents. In another variation, the assay strip 190 may not be prepackaged with a set of molecular diagnostic reagents, and the wells 193 of the assay strip 190 may not be sealed with a 50 puncturable foil seal 195. In yet another variation, the system may comprise an empty assay strip 190 without a puncturable foil seal 195, and an assay strip 190 comprising reagents and a puncturable foil seal 195, such that a user may add specific reagents to the empty assay strip to be used in 55 conjunction with the assay strip comprising reagents. In variations comprising a puncturable foil seal 195, the puncturable foil seal 115 is configured to be punctured by at least one external element, for co-delivery of nucleic acid samples and molecular diagnostic reagents intended to be combined. 60

In a specific example, the assay strip **190** has an 87 mm×16 mm footprint and comprises 24 wells **113** arranged in two staggered rows, with a 9 mm center-to-center pitch between adjacent wells **193** within each row. Each well **193** of the set of wells has a capacity of  $60 \mu L$  to accommodate 65 a volume of a molecular diagnostic reagent, 20  $\mu L$  of a sample fluid, and any displacement caused by a pipette tip

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(e.g., 100 or  $300 \,\mu\text{L}$  pipette tip). Each well **113** of the assay strip **190** in the specific example is also prepackaged with a quantity of molecular diagnostic reagents, and comprises a protruding top edge (75 microns high) that is heat sealed to a puncturable foil seal. The capture plate **110** in the specific example is produced by injection molding, has a footprint of 127.75 mm×85.5 mm, and is composed of a PCR-compatible polypropylene based polymer with a high vapor barrier. In the specific embodiment, the vapor barrier is further increased by depositing a thin metallic layer to the outside of the assay strip **190**.

As described earlier, the assay strip 190 may be configured to be received by an assay strip holder 230. The assay strip holder 230 functions to receive and align multiple assay strips 190, such that a multichannel pipettor or other fluid delivery system may combine multiple nucleic acid samples with molecular diagnostic reagents using wells 193 of multiple assay strips 190. In one variation, the assay strip holder 230 may be configured to contain Assay strips 190 including reagents for substantially different molecular diagnostic assays, as shown in FIG. 17B, such that a single run of the system 100 involves analyzing a set of nucleic acid samples under different molecular diagnostic assays. In another variation, the assay strip holder 230 may be configured to contain assay strips 190 including reagents for identical molecular diagnostic assays, such that a single run of the system 100 involves analyzing a set of nucleic acid samples under the same molecular diagnostic assay. Preferably, the assay strip holder 230 is composed of a material that is dishwasher safe and autoclavable, configured to hold the assay strips 190 in place during handling by a fluid delivery system (e.g., pipettor), and configured such that the assay strips 190 avoid protruding over an edge of the assay strip holder 230, but the assay strip holder 230 is constructed to facilitate insertion and removal of the assay strips 190 from the assay strip holder 230.

In one variation, the assay strip holder 230 is not configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190; however, in another variation as shown in FIG. 21A, the assay strip holder 230 may be further configured to couple to an aluminum block 235 coupled to a set of Peltier units 236 configured to facilitate cooling of molecular diagnostic reagents within the assay strips 190. Additionally, the assay strip holder 230 may be configured to be received and carried by an assay strip carrier 240, which, as shown in FIG. 20, functions to facilitate handling and alignment of multiple assay strip holders 230. In a specific example, as shown in FIG. 19, the assay strip holder 230 has dimensions of 127.76 mm×85.48 mm×14.35 mm, complies with American National Standards Institute (ANSI) and Society for Laboratory Automation and Screening (SLAS) standards, and is configured to hold six 16-well assay strips for a total of 96 wells 193. In another specific example, as shown in FIG. 21B, the assay strip holder 230' is configured to hold four assay strips 190', each comprising 24 wells 193' for a total of 96 wells per assay strip holder 230'. Other combinations of the described embodiments, variations, and examples of the assay strip 190, assay strip holder 230, and assay strip carrier 240 may be incorporated into embodiments of the system 100 for processing and detecting nucleic acids.

1.4 System—Microfluidic Cartridge

The microfluidic cartridge 210 functions to receive a set of magnetic bead-samples, facilitate separation of nucleic acids from the set of magnetic bead-samples, receive a set of nucleic acid-reagent samples, and facilitate analysis of nucleic acids from the set of nucleic acid-reagent samples.

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In one embodiment, the microfluidic cartridge 210 comprises a top layer 211 including a set of sample port-reagent port pairs 212 and a set of detection chambers 213; an intermediate substrate 214, coupled to the top layer 211 and partially separated from the top layer 211 by a film layer 215, 5 configured to form a waste chamber 216; an elastomeric layer 217 partially situated on the intermediate substrate 214; a magnet housing region 218 accessible by a magnet 160 providing a magnetic field; and a set of fluidic pathways 219, each formed by at least a portion of the top layer 211, 10 a portion of the film layer 215, and a portion of the elastomeric layer 217. In the embodiment, the microfluidic cartridge 10 further comprises a bottom layer 221 coupled to the intermediate substrate 214 and configured to seal the waste chamber 216. Furthermore, in the embodiment, the 15 top layer 211 of the microfluidic cartridge 210 further comprises a shared fluid port 222, a vent region 223, and a heating region 224, such that each fluidic pathway 220 in the set of fluidic pathways 219 is fluidically coupled to a sample port-reagent port pair 224, the shared fluid port 222, the 20 waste chamber 216, and a detection chamber 225, comprises a turnabout portion 226 configured to pass through the heating region 224 and the magnetic field, and is configured to pass through the vent region 223 upstream of the detection chamber 225. Each fluidic pathway 220 thus functions to 25 receive and facilitate processing of a sample fluid containing nucleic acids as it passes through different portions of the fluidic pathway 220.

The microfluidic cartridge 210 is preferably configured to be received and manipulated by the molecular diagnostic 30 module 130, such that the cartridge receiving module 140 of the molecular diagnostic module 130 receives and aligns the microfluidic cartridge 210 within the molecular diagnostic module 130, the heating and cooling subsystem 150 of the molecular diagnostic module 130 is configured to transfer 35 heat to the heating region 224 of the microfluidic cartridge 210, and the magnet 160 of the molecular diagnostic module 130 is configured to be received by the magnet housing region 218 of the microfluidic cartridge 210 to provide a magnetic field for separation of nucleic acids. Additionally, 40 the shared fluid port 222 of the microfluidic cartridge 210 is configured to couple to a nozzle 149 coupled to the linear actuator 146 of the cartridge receiving module 140, such that the liquid handling system 250 can deliver fluids and gases through the shared fluid port 222. The elastomeric layer 217 45 of the microfluidic cartridge 210 is also preferably configured to be occluded at a set of occlusion positions 226 by the valve actuation subsystem 170 of the molecular diagnostic module, in order to occlude portions of a fluidic pathway 220 of the microfluidic cartridge 210 for processing of a set 50 of biological samples. The optical subsystem 180 of the molecular diagnostic module 130 is further configured to align with the set of detection chambers 213 of the microfluidic cartridge 210, to facilitate analysis of a set of nucleic acid samples. The microfluidic cartridge 210 is preferably 55 the microfluidic cartridge 210 described in U.S. application Ser. No. 13/765,996, which is incorporated in its entirety by this reference, but may alternatively be any appropriate cartridge or substrate configured to receive and process a set of samples containing nucleic acids.

#### 1.5 System—Fluid Handling System and Filter

The liquid handling system 250 of the system 100 includes a liquid handling arm 255 and a syringe pump 265, as shown in FIGS. 14A-14C and functions to deliver biological samples, reagents, and gases to elements of the 65 system 100. As described in Section 1, an embodiment of the liquid handling system 250 is configured to aspirate a set of

biological samples containing nucleic acids (i.e., impure nucleic acid samples), dispense the set of biological samples into a capture plate 110 to be lysed and combined with magnetic beads by a capture plate module 120, aspirate the set of biological samples combined with magnetic beads (i.e., set of magnetic bead-samples) from the capture plate 110, and dispense the set of magnetic bead-samples into microfluidic cartridge 210 located in a molecular diagnostic module 130. The embodiment of the liquid handling system 100 is further configured to facilitate separation of a set of nucleic acids from the magnetic bead-samples, by dispensing a wash solution, a release solution, and/or air into the molecular diagnostic module 130, by the nozzle 149 coupled to the linear actuator 146, at appropriate stages, aspirate the set of nucleic acids from the molecular diagnostic module 130, combine the set of nucleic acids with a set of molecular diagnostic reagents using an assay strip 190, and dispense the set of nucleic acids combined with the set of molecular diagnostic reagents (i.e., set of nucleic acid-reagent mixtures) into the molecular diagnostic module 130 for further processing and analysis. [Other embodiments of the liquid handling system 250 may be configured to perform alternative molecular diagnostic assay protocols and/or dispense and aspirate alternative fluids into and from other elements supporting a molecular diagnostic protocol.

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The liquid handling arm 255 comprises a gantry 256 and a multichannel liquid handling head 257, and functions to travel to different elements of the system 100 for fluid delivery and aspiration. The liquid handling arm 255 is preferably automated and configured to move, aspirate, and deliver fluids automatically, but may alternatively be a semi-automated liquid handling arm 255 configured to perform at least one of moving, aspirating, and delivering automatically, while another entity, such as a user, performs the other functions.

The gantry 256 is coupled to the multichannel liquid handling head 257, and functions to transport the multichannel liquid handling head 257 to different elements of the system 100 for fluid delivery and aspiration. Preferably, the gantry 256 is automated and configured to translate the multichannel liquid handling head 257 within at least two dimensions, and provides X-Y positional accuracy of at least 0.5 mm. Additionally, in the orientation shown in FIG. 14B, the gantry is preferably situated above the molecular diagnostic module 130, such that the gantry 256 can translate within at least two dimensions without interfering with other elements of the system 100. Alternatively, the gantry 256 may be any appropriate gantry 256 to facilitate movement of an end effector within at least two dimensions, as is readily known by those skilled in the art.

The multichannel liquid handling head 257 functions to aspirate fluids from and deliver fluids to different elements of the system 100. Preferably, the multichannel liquid handling head 257 is a multichannel pipette head; however, the multichannel liquid handling head 257 may alternatively be any appropriate multichannel liquid handling head configured to deliver fluids and/or gases. Preferably, the multichannel liquid handling head 257 comprises at least eight independent channels 258, but may alternatively comprise 60 any number of channels 258 configured to aspirate and deliver fluids. The channel-to-channel pitch is preferably variable, and in a specific example ranges between 9 mm and 36 mm; however, the channel-to-channel pitch may alternatively be fixed, as shown in FIG. 15. The multichannel liquid handling head 257 also preferably provides independent z-axis control (in the orientation shown in FIG. 14B), such that, in combination with the gantry 256. The

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multichannel liquid handling head 257 is preferably configured to couple to both large (e.g., 1 mL) and small (e.g., between 100 and 300 µL) pipette tips, and in a specific example, has a precision of at least 6% using small disposable pipette tips and a precision of at least 2% using large 5 disposable pipette tips when dispensing essentially the entire tip volume. Alternatively, the multichannel liquid handling head 257 may be configured to couple to any object configured to facilitate aspiration and delivery of fluids. Preferably, the multichannel liquid handling head 257 provides independent control of the channels 258, with regard to volumes of fluid aspirated or delivered, fluid dispensing rates, and/or engaging and disengaging pipette tips. Alternatively, the multichannel liquid handling head 257 may not provide independent control of the channels 258, such that 15 all channels 258 of the multichannel liquid handling head 257 are configured to perform identical functions simultaneously. Preferably, the multichannel liquid handling head 257 is configured to aspirate and deliver both liquids and gases, but alternatively, the multichannel liquid handling 20 head 257 may be configured to only aspirate and deliver liquids. Preferably, the multichannel liquid handling head 257 provides at least one of liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258; however, the multichannel liquid 25 handling head 257 may alternatively not provide liquid level detection, clot detection, and pipette tip engaging/disengaging detection for each of the channels 258.

In one embodiment, the multichannel liquid handling head 257 is configured to couple to at least one filter 260, 30 which functions to pre-filter liquids being aspirated and/or dispensed by the liquid handling arm 255, and is preferably a custom filter 260 configured to couple to a pipette tip, but may alternatively be any appropriate filter configured to couple to the liquid handling arm 255 and filter liquids being 35 aspirated and/or dispensed by the liquid handling arm 255.

An embodiment of a custom filter 260, as shown in FIG. 22, comprises a first end 261 configured to couple to a pipette tip, a pointed second end 262, a void 263 coupled to the first end 261 and the pointed second end 262, and a filter 40 membrane 264 subdividing the void 263. The first end 261, as shown in FIG. 22, preferably comprises a tapered channel configured to provide a friction fit with a pipette tip; however, the first end may alternatively not comprise a tapered channel and may be configured to couple to a pipette tip 45 using any appropriate means. The pointed second end 262 is preferably sharp and configured to pierce an object, such as a foil seal; additionally, the pointed second end 262 is preferably at least as long as required to dispense into a well 113 of the capture plate 110. The void 263 preferably defines 50 a conical region defined by the filter membrane 264, wherein the conical region is configured to divert a fluid within the filter 260 toward the pointed second end 262; however, the void 263 may not include a conical region. The filter membrane 264 functions to filter a fluid aspirated by the 55 multichannel liquid handling head 257, and is configured to subdivide the void 263 to define a conical region; however, the filter membrane 264 may alternatively not define a conical region of the void 263. In one embodiment, in the orientation shown in FIG. 22, the region of the void 263 60 below the filter membrane 264 may have a volumetric capacity of between 200 ul and 1 mL; however, the region of the void 263 below the filter membrane may alternatively have any appropriate volumetric capacity.

A set of filters **260** may further be configured to be 65 received and delivered by a filter holder **269**, as, shown in FIG. **23**. A specific embodiment of a filter holder **269** 

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comprises a set of 24 tapered holes with an 18 mm center-to-center pitch, arranged in six rows of four holes. The specific embodiment of the filter holder **269** is also compliant with ANSI and SLAS standards, has dimensions of 127.75×85.5×14.35 mm, and is stackable with other specific embodiments of the custom filter holder **269**. Alternatively, the filter holder **269** may be any appropriate filter holder **269** configured to receive and deliver a set of filters **260**, as is readily known by those skilled in the art.

1.5.1 Fluid Handling System—Syringe Pump

The syringe pump 265 of the liquid handling system 250 is coupled to a wash solution source 266, a release solution source 267, a source of air 268, and flexible tubing 291, and functions to deliver a wash solution, a release solution, and air through a valve to the molecular diagnostic module 130 to facilitate isolation and purification of nucleic acids from a set of magnetic bead-samples. The flexible tubing 291 is preferably coupled at a first end to the syringe pump, and at a second end to a nozzle 149 coupled to the linear actuator 146 of the molecular diagnostic module 130, as shown in FIG. 14C. As stated earlier, an extended configuration 146b of the linear actuator 146 is configured to couple the nozzle 149 to a fluid port 222 of a microfluidic cartridge 210 within the molecular diagnostic module 130, such that the wash solution, release solution, and air can be delivered to the microfluidic cartridge 210 at appropriate stages. A specific embodiment of the syringe pump 265 comprises a 4-way valve, is able to pump 20-5000 μL of fluids or air through the 4-way valve at flow rates from 50-500 μL/min, can couple to syringes with between 1 mL and 10 mL capacities, and has a precision of at least 5% with regard to fluid or air delivery. Alternatively, the syringe pump 265 may be any appropriate syringe pump 265 or fluid delivery apparatus configured to deliver a wash solution, a release solution, and air to the molecular diagnostic module 130, as is readily known by those skilled in the art.

1.6 System—Additional Elements

The system 100 may further comprise a tag reader 271, which functions to read barcodes, QR codes and/or any other identifying tags of the system 100. Preferably, the tag reader 271 is coupled to the liquid handling system 250, such that the tag reader 271 is configured to read tags on puncturable foil seals 115, 195 or tags located on any element of the system 100 accessible by the liquid handling system 250; however, the tag reader 271 may alternatively not be coupled to the liquid handling system 250. In one alternative embodiment of the system 100, the tag reader 271 may be a standalone unit that is configured to be manipulated by a user to scan tags or labels located on elements of the system 100.

The system 100 may also further comprise a controller 272 coupled to at least one of the capture plate module 120, the molecular diagnostic module 130, the liquid handling system 250, and the tag reader 271, and functions to facilitate automation of the system 100. In a variation wherein the controller 272 is coupled to the capture plate module 120, the controller 272 preferably functions to automate heating of a capture plate 110, which facilitates lysing of biological samples within the capture plate 110 and binding of nucleic acids within the capture plate 110 to magnetic beads 119 of the capture plate 110. In a variation wherein the controller 272 is coupled to the molecular diagnostic module 130, the controller 272 preferably functions to automate reception of a microfluidic cartridge, heating of biological samples within the molecular diagnostic module 130 and the detection chambers 213, occlusion of fluidic pathways 220 by the valve actuation subsystem 170, and analysis of a set of

nucleic acid-reagent mixtures by the optical subsystem 180. In a variation wherein the controller 272 is coupled to the liquid handling system 250, the controller 272 preferably functions to automate aspiration, transfer, and delivery of fluids and/or gases to different elements of the system 100. 5 In a variation wherein the controller 272 is coupled to the tag reader 271, the controller preferably functions to automate reading of tags by the tag reader 271, and may further function to facilitate transfer of information from the tags to a processor 273. Other variations of a controller may func- 10 tion automate handling, transfer, and/or storage of other elements of the system 100, such as capture plates 110, assay strips 190, assay strip holders 230, assay strip carriers 240, filters 200, filter holders 205, and/or microfluidic cartridges 210, using a robotic arm or gantry similar to that used in the 15 liquid handling system 250. Alternative combinations of the above variations may involve a single controller 272, or

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The system 100 may also further comprise a processor 20 273, which functions to receive and process information from a tag reader 271, and also to receive and process data received from the optical subsystem 180 of the molecular diagnostic module 130. Preferably, the processor 273 is coupled to a user interface 274, which functions to display 25 processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information. Alternatively, the processor 273 is not coupled to a user interface 274, but comprises a connection 275 configured to 30 facilitate transfer of processed and/or unprocessed data produced by the system 100, settings of the system 100, information obtained from a tag reader 271, or any other appropriate information to a device external to the system 100.

multiple controllers configured to perform all or a subset of

the functions described above.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made the described embodiments of the system 100 without departing from the scope of the system 100.

2. Method for Processing and Detecting Nucleic Acids

An embodiment of a method 400 for processing and detecting nucleic acids from a set of biological samples comprises: combining each biological sample of the set of biological samples with a quantity of magnetic beads to 45 produce a set of magnetic bead-sample mixtures S410; heating the set of magnetic bead-sample mixtures to produce a set of nucleic acid-magnetic bead samples S420; transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding 50 fluidic pathway of a set of fluidic pathways S430; producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples S440; combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic 55 reagents to produce a set of nucleic acid-reagent mixtures S450; transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers S460; and receiving light from the set of 60 nucleic acid-reagent mixtures S470. The method 400 may further comprise generating a set of data based on light received form the set of nucleic acid-reagent mixtures S480. The method 400 functions to isolate and extract a set of nucleic acid volumes from a biological sample, and to 65 facilitate analysis of the nucleic acid volumes according to at least one molecular diagnostic protocol.

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Step S410 recites combining each biological sample of the set of biological samples with a quantity of magnetic beads to produce a set of magnetic bead-sample mixtures, and functions to prepare a set of biological samples to be lysed and combined with magnetic beads. For each biological sample, Step S410 preferably comprises aspirating a portion of the volume of the biological sample from a sample container (possibly containing an aqueous solution prior to addition of biological sample), and transferring the portion of the biological sample to a well containing a set of magnetic beads. Alternatively, for each biological sample, Step S410 may comprise aspirating the entire volume of the biological sample from a sample container, and transferring the volume of the biological sample to be combined with a set of magnetic beads. Preferably, all biological samples in the set of biological samples are aspirated and combined with the magnetic beads in the wells simultaneously using a multichannel fluid delivery system; however, all biological samples in the set of biological samples may alternatively be aspirated and combined with a set of magnetic beads nonsimultaneously. The magnetic beads are preferably polymer beads, precoupled with a ligand for binding to a nucleic acid, and comprising a superparagmagnetic component. Additionally, the magnetic beads may be treated to be positively charged. However, the magnetic beads may alternatively be any appropriate magnetic beads configured to facilitate biomagnetic separation.

In addition to combination with magnetic beads, Step 410 may further include combining each biological sample of the set of biological samples with a lysing enzyme (e.g. proteinase K), and a sample process control comprising two or more nucleic acid sequences (i.e., one for DNA and one for RNA) to be included with each sample. This allows biological samples to effectively lysed, which releases waste components into a wash solution, and allows nucleic acids to bind to magnetic beads. This additionally allows the sample process control to be later detected, as a check to verify the accuracy of a molecular diagnostic assay being performed.

In a first variation of Step S410 for one biological sample, as shown in FIG. 16A, a volume of the biological sample is aspirated and combined with a set of magnetic beads. In the first variation of Step S410, a set of different biological samples may thus be aspirated simultaneously, and each biological sample may be transferred to an individual well to be combined with a set of magnetic beads to produce a set of magnetic bead-sample mixtures. In the first variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic bead-sample mixtures are substantially non-identical in composition. In a second variation of Step S410, as shown in FIG. 16B, a volume of a stock biological sample is aspirated, and portions of the volume of the stock biological sample are transferred to multiple wells to be combined with multiple sets of magnetic beads to produce a set of magnetic bead-sample mixtures. In the second variation of Step S410, all magnetic bead-sample mixtures in the set of magnetic bead-sample mixtures are substantially identical in composition. Other variations of Step S410 may comprise filtering at least one biological sample of the set of biological samples S415 prior to combining each biological sample of the set of biological samples with a quantity of magnetic

In a specific example of Step S410, a multichannel liquid handling system aspirates approximately 1 mL of each of a set of biological samples in aqueous buffer using a set of 1 mL pipette tips, couples each of the pipette tips to a custom 13 mm diameter filter, punctures a foil seal 115 of a capture

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plate at a set of wells, wherein each well of the set of wells contains a set of magnetic beads, and dispenses each aspirated volume of a biological sample into a well of the capture plate containing a set of magnetic beads, and disposes of the tip/filter combination. In the specific example of 5 Step S410, the multichannel liquid handling system then picks up new disposable tips and aspirates and dispenses the contents of each well at least three times to mix the contents, and then disposes of the set of pipette tips and filters.

Step S420 recites heating the set of magnetic bead-sample 10 mixtures to produce a set of nucleic acid-magnetic bead samples, and functions to incubate the set of magnetic bead-sample mixtures in order to lyse biological matter, and release nucleic acids to be bound to magnetic beads. Preferably, Step S420 comprises heating a capture plate con- 15 taining the set of magnetic bead-sample mixtures for a specified amount of time at a specified temperature, and may additionally include cooling the set of magnetic bead-sample mixtures. In a specific example, Step S420 comprises heating a capture plate containing the set of magnetic bead- 20 sample mixtures using a capture plate module, wherein the capture plate module is configured to cradle and controllably heat wells containing the set of magnetic bead-sample mixtures. Step S420 may alternatively comprise incubating the set of magnetic bead-sample mixtures using any appro- 25 priate method and/or system as is known by those skilled in the art. Finally, Step S420 may be omitted in embodiments of the method 400 involving samples that do not require heating.

Step S430 recites transferring each nucleic acid-magnetic 30 bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways, and functions to isolate each of the set of nucleic acid-magnetic bead samples within separate pathways for further processing. Preferably, all nucleic acid-magnetic 35 bead samples in the set of nucleic acid-magnetic bead samples are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acid-magnetic bead sample in the set of magnetic bead-samples may be transferred to a corresponding fluidic pathway independently of 40 the other nucleic acid-magnetic bead samples. In addition, preferably the entire volume, or substantially all of the volume, of the nucleic acid-magnetic bead sample is transferred to the set of fluidic pathways, without magnetically isolating magnetic beads and removing supernatant fluids 45 prior to transferring each nucleic acid-magnetic bead sample of the set of nucleic acid-magnetic bead samples to a corresponding fluidic pathway of a set of fluidic pathways.

Step S430 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of 50 a set of occlusion positions S432, which functions to define at least one truncated fluidic pathway. Preferably, Step S432 comprises defining at least one truncated fluidic pathway passing through at least one of a heating region and a magnetic field; however, Step S432 may alternatively not 55 comprise defining a truncated fluidic pathway passing through at least one of a heating region and a magnetic field.

In a specific example of Step S430, the multichannel liquid handling subsystem of Step S410 transfers a set of nucleic acid-magnetic bead samples to a set of fluidic 60 pathways of a microfluidic cartridge aligned within a molecular diagnostic module, wherein the microfluidic cartridge comprises an elastomeric layer in contact with the set of fluidic pathways. Manipulation of the elastomeric layer at a subset of a set of occlusion positions by a valve actuation 65 subsystem of the molecular diagnostic module defines a set of truncated fluidic pathways crossing a heating region and

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a magnetic field, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a truncated fluidic pathway of the set of truncated fluidic pathways.

Step S440 recites producing a set of nucleic acid volumes from the set of nucleic acid-magnetic bead samples, and functions to separate nucleic acid volumes from the set of nucleic acid-magnetic bead samples. Step S440 preferably reduces a concentration of unwanted matter from the set of biological samples being processed, to an acceptable level; however, Step S440 may alternatively entirely remove substantially all unwanted substances from the set of biological samples being processed. Step S440 preferably includes providing a magnetic field S441, such that each fluidic pathway in the set of fluidic pathways is configured to cross the magnetic field. Preferably, the set of nucleic acidmagnetic bead samples is captured and isolated within portions of the set of fluidic pathways crossing the magnetic field. Step S440 may further comprise providing a heater configured to span a heating region of the set of fluidic pathways S442, but may alternatively comprise providing multiple heaters or altogether omit providing a heater. In embodiments wherein multiple heaters are provided, each heater is preferably independent to allow independent control of heating time and temperature for each sample. Step S442 functions to provide a heater, which, in combination with a release solution that provides a pH shift, facilitate a rapid and efficient unbinding of the nucleic acids from magnetic beads.

Step S440 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S443 (and opening a previously occluded channel), which functions to define at least one truncated fluidic pathway containing a nucleic acid-magnet bead sample and coupled to a source for delivery of a wash solution and a release solution. Preferably, Step S443 comprises defining at least one truncated fluidic pathway coupled to a waste chamber and to a fluid port, which functions to facilitate washing of at least one nucleic acidmagnetic bead sample in the set of nucleic acid-magnetic bead samples, and releasing of at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. Step S440 may additionally comprise delivering a wash solution through a portion of at least one fluidic pathway S444, such as the truncated fluidic pathway defined in Step S443, and delivering a release solution through a portion of at least one fluidic pathway S445, such as the truncated fluidic pathway defined in Step S443. Step S444 functions to wash at least one nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples, and Step S445 functions to release at least one nucleic acid volume from the set of nucleic acid-magnetic bead samples. The heater provided in Step S442 may be activated after Step S445 to induce a pH shift.

In a specific example of Step S440, the set of fluidic pathways containing a set of nucleic acid-magnetic bead samples, from the specific example of Step S430, is occluded at a subset of the set of occlusion positions by a valve actuation subsystem of the molecular diagnostic module, to define a set of truncated fluidic pathways coupled to a waste chamber and to a shared fluid port of the microfluidic cartridge for delivery of a wash solution and a release solution. The liquid handling system delivers a wash fluid through the shared fluid port to wash the set of nucleic acid-magnetic bead samples, captured within the magnetic field, and then delivers a release fluid through the shared fluid port to release a set of nucleic acid volumes from the

set of nucleic acid-magnetic bead samples. In the specific example, each fluidic pathway is washed sequentially, and the release solution is delivered to each fluidic pathway sequentially to ensure that each lane is provided with substantially equal amounts of wash and release solutions. All waste fluid produced in the specific example of Step S440 pass into the waste chamber coupled to the set of truncated fluidic pathways.

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Step S450 recites combining each nucleic acid volume of the set of nucleic acid volumes with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures, which functions to prepare the set of nucleic acid volumes to be detected. For each nucleic acid volume in the set of nucleic acid volumes, 15 Step S450 preferably comprises aspirating an entire volume of the nucleic acid volume from its corresponding fluidic pathway, and transferring the nucleic acid volume to a well containing a molecular diagnostic reagent. Preferably, all nucleic acid volumes in the set of nucleic acid volumes are 20 aspirated and combined with molecular diagnostic reagents simultaneously using a multichannel fluid delivery system; however, each nucleic acid volume in the set of nucleic acid volumes may alternatively be aspirated and combined with molecular diagnostic reagents independently of the other 25 nucleic acid volumes. The molecular diagnostic reagents preferably comprise reagents configured to analyze the set of nucleic acid volumes for markers of at least one of gonorrhea (GC), Chlamydia (CT), herpes simplex virus (HSV), human immunodeficiency virus (HIV), human respiratory 30 diseases, vaginal diseases, hepatitis C virus (HCV), hepatitis B virus (HBV), trichonomas, group B streptococcus (GBS), factor 2 (FII) gene, and factor five (FV) gene, but may alternatively comprise reagents used to detect any specific nucleic acid sequence.

In a first variation of Step S450 as shown in FIG. 16A, a nucleic acid volume is aspirated and combined with a molecular diagnostic reagent for a single assay. In the first variation of Step S450, a set of nucleic acid volumes may thus be aspirated simultaneously, and each nucleic acid 40 volume may be transferred to an individual well to be combined with a molecular diagnostic reagent of a set of molecular diagnostic reagents to produce a set of nucleic acid-reagent mixtures. In the first variation of Step S450, all nucleic acid-reagent mixtures in the set of nucleic acid- 45 reagent mixtures may or may not be substantially identical in composition, depending on the homogeneity of the biological samples used in Step S410; however, the first variation of S450 preferably comprises using identical molecular diagnostic reagents, such that identical molecular diagnostic 50 protocols analyzing identical markers may be performed. Thus, the first variation of Step S450 encompasses running multiple identical tests from a stock biological sample (e.g., a multiplex assay), and running identical tests using a set of substantially different biological samples (e.g., from differ- 55 ent sources).

In a second variation of Step S450, as shown in FIG. 16B, the set of nucleic acid volumes is aspirated, and each nucleic acid volume in the set of nucleic acid volumes is combined with a molecular diagnostic reagent of a set of molecular 60 diagnostic reagents. In the second variation of Step S450, the set of molecular diagnostic reagents preferably comprises different molecular diagnostic reagents, such that different molecular diagnostic protocols analyzing different markers may be performed. Thus, the second variation 65 encompasses running multiple substantially different tests using a stock biological sample, and running substantially

different tests using substantially different biological samples (e.g., from different sources)

In a specific example of Step S450, a multichannel liquid handling system aspirates approximately 18  $\mu$ L of each of a set of nucleic acid volumes from the microfluidic cartridge used in the specific example of Step S440 using a set of pipette tips, punctures at least one foil seal 195 of at least one assay strip, wherein each well of the at least one assay strip contains molecular diagnostic reagents, and dispenses each aspirated nucleic acid volume into a well of the assay strip. In the specific example of S450, the multichannel liquid handling system then aspirates and dispenses the contents of each well approximately 10 times to reconstitute molecular diagnostic reagents and mix the contents of each well.

Step S460 recites transferring each of the set of nucleic acid-reagent mixtures, through the corresponding fluidic pathway of the set of fluidic pathways, to a detection chamber of a set of detection chambers, which functions to deliver the set of nucleic acid-reagent mixtures to an isolated detection chamber for further processing and analysis. Preferably, all nucleic acid-reagent mixtures in the set of nucleic acid-reagent mixtures are transferred simultaneously to the set of fluidic pathways, but alternatively, each nucleic acidreagent mixture in the set of nucleic acid reagent mixtures may be transferred to a corresponding fluidic pathway independently of the other nucleic acid reagent mixtures. Step S460 may further comprise occluding at least one fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions S462, which functions to define at least one truncated fluidic pathway coupled to a detection chamber of a set of detection chambers. Preferably, Step S462 comprises occluding each fluidic pathway of the set of fluidic pathways at a subset of a set of occlusion positions, thus defining a set of truncated fluidic pathways, each 35 coupled to a detection chamber.

In a specific example of Step S460, the multichannel liquid handling subsystem of the specific example of Step S450 transfers a set of nucleic acid-reagent mixtures, each having a volume of approximately 16 µL, back to the set of fluidic pathways of the microfluidic cartridge of the specific example of Step S450. Each nucleic acid-reagent mixture in the set of nucleic acid-reagent mixtures is transferred at a rate of 50 μL/minute. Manipulation of the elastomeric layer at a subset of a set of occlusion positions by the valve actuation subsystem of the molecular diagnostic module defines a set of truncated fluidic pathways, each coupled to a detection chamber, such that each nucleic acid-magnetic bead sample in the set of nucleic acid-magnetic bead samples is isolated within a truncated fluidic pathway of the set of truncated fluidic pathways. In the specific embodiment the occlusion position immediately upstream of the detection chamber and the occlusion position immediately downstream of the detection chamber are normally closed positions. During delivery, the multichannel liquid handling subsystem generates pressure to cause the elastomeric layer at the normally closed positions to deform and allow fluid to flow through the normally closed positions. Once the pressure drops after the detection chamber is filled and the multichannel liquid handing subsystem ceases delivery, the elastomeric layer is configured to overcome the pressure in the channel and recloses, thereby sealing the normally closed positions. The normally closed positions are then compressed using the valve actuation subsystem during thermocycling to prevent pressures generated during a molecular diagnostic assay to cause the normally closed positions to leak. After the molecular diagnostic assay is complete and the occlusion "pins" withdrawn, the normally

closed positions allow the samples and amplicons to be trapped within detection chambers, substantially reducing the risk of contamination of the lab or other samples.

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Step S470 recites receiving light from the set of nucleic acid-reagent mixtures, and functions to produce emission 5 responses from the set of nucleic acid-reagent mixtures in response to transmission of excitation wavelength light or chemiluminescent effects. Preferably, Step S470 comprises the ability to transmit light including a wide range of wavelengths through a set of excitation filters and through a set of apertures configured to individually transmit light having single or multiple excitation wavelengths onto the set of nucleic acid-reagent mixtures, and receiving light through a set of emission filters, from the set of nucleic acid-reagent mixtures. Step S470 may additionally comprise reflecting light from the set of excitation filters off of a set of dichroic mirrors, and transmitting light through the set of dichroic mirrors to a set of photodetectors. A specific example of Step S470 comprises using the optical subsystem 180 of the 20 system 100 described above to transmit and receive light; however, alternative variations of Step S470 may use any appropriate optical system configured to transmit light at excitation wavelengths toward the set of nucleic acid-reagent mixtures, and to receive light at emission wavelengths 25 from the set of nucleic acid-reagent mixtures.

Step S480 recites generating a set of data based on light received from the set of nucleic acid-reagent mixtures, which functions to produce quantitative and/or qualitative data from the set of nucleic acid-reagent mixtures. Step S480 30 may further function to enable detection of a specific nucleic acid sequence from the nucleic acid-reagent mixture, in order to identify a specific nucleic acid sequence, gene, or organism. Preferably, Step S480 includes converting electrical signals, produced by a set of photodetectors upon 35 receiving light from the set of nucleic acid-reagent mixtures, into a quantifiable metric; however, S480 may alternatively comprise converting electromagnetic energy, received by a set of photodetectors from the set of nucleic acid-reagent mixtures, into a set of qualitative data. In one variation of 40 Step S480, the set of data may be processed by a processor and rendered on a user interface; however, in other variations of Step S480, the set of data may alternatively not be rendered on a user interface.

The method **400** may further comprise re-running a 45 biological sample S**490** if processing and/or analysis of the biological sample results in less than ideal results. Preferably, Step S**490** occurs if an analysis of a biological sample is indeterminate due to machine or user error. Additionally, Step S**490** preferably occurs automatically upon detection of 50 a less than ideal result, but may alternatively occur in response to a user prompt.

Embodiments of the method 400 and variations thereof can be embodied and/or implemented at least in part by a machine configured to receive a computer-readable medium 55 storing computer-readable instructions. The instructions are preferably executed by computer-executable components preferably integrated with the system 100 and one or more portions of the processor 273 and/or the controller 272. The computer-readable medium can be stored on any suitable 60 computer-readable media such as RAMs, ROMs, flash memory, EEPROMs, optical devices (CD or DVD), hard drives, floppy drives, or any suitable device. The computer-executable component is preferably a general or application specific processor, but any suitable dedicated hardware or 65 hardware/firmware combination device can alternatively or additionally execute the instructions.

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The FIGURES illustrate the architecture, functionality and operation of possible implementations of systems, methods and computer program products according to preferred embodiments, example configurations, and variations thereof. In this regard, each block in the flowchart or block diagrams may represent a module, segment, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that, in some alternative implementations, the functions noted in the block can occur out of the order noted in the FIGURES. For example, two blocks shown in succession may, in fact, be executed substantially concurrently, or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved. It will also be noted that each block of the block diagrams and/or flowchart illustration, and combinations of blocks in the block diagrams and/or flowchart illustration, can be implemented by special purpose hardware-based systems that perform the specified functions or acts, or combinations of special purpose hardware and computer instructions.

As a person skilled in the art will recognize from the previous detailed description and from the figures and claims, modifications and changes can be made to the preferred embodiments of the invention without departing from the scope of this invention defined in the following claims.

We claim:

- 1. A molecular diagnostic system configured to process a biological sample within a cartridge and separate a nucleic acid volume from the biological sample, the molecular diagnostic system comprising:
  - a cartridge platform that supports the cartridge and comprising a magnet receiving slot configured to be aligned with the cartridge in a first operation mode;
  - a nozzle of a liquid handling subsystem;
  - an optical subsystem;
  - a cartridge heater;
  - a magnet vertically aligned with the magnet receiving slot; and
  - an actuator coupled to the nozzle of the liquid handling subsystem, the optical subsystem, and the cartridge heater, the actuator configured to vertically displace the cartridge platform in the first operation mode to a position wherein:
    - the nozzle of the liquid handling system is coupled to a fluid port of the cartridge, wherein the fluid port of the cartridge receives fluids for processing the biological sample,
    - the magnet passes through the magnet receiving slot of the cartridge platform and interfaces with a first portion of the cartridge,
    - the optical subsystem interfaces with a second portion of the cartridge, wherein the second portion of the cartridge receives a processed derivative of the nucleic acid volume, and
    - a third region of the cartridge is compressed between the cartridge heater and the cartridge platform.
  - 2. The system of claim 1, further including:
  - a valve actuation subsystem comprising a set of pins, wherein the actuator vertically displaces the cartridge platform in the first operation mode to a position where the cartridge is positioned proximal the set of pins.
- 3. The system of claim 2, wherein in the first operation mode, the actuator provides a counterforce opposing the valve actuation subsystem, in cooperation with manipulation of the cartridge using the set of pins of the valve actuation subsystem.

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- 4. The system of claim 2, further comprising:
- a cam module configured to displace a subset of the set of pins of the valve actuation subsystem in the first operation mode to define a pathway, wherein the pathway facilitates separation of the nucleic acid volume 5 from the biological sample, within the cartridge.
- 5. The system of claim 4, wherein the cam module includes a cam card coupled to a cam card actuator configured to linearly displace the cam card in interfacing with the set of pins.
- 6. The system of claim 5, wherein the cam card comprises a fixed set of hills and valleys, such that a pin of the set of pins is in a first position when a hill of the cam card interfaces with the pin, and the pin of the set of pins is in a second position when a valley of the cam card interfaces 15 with the pin.
- 7. The system of claim 6, wherein at least one pin in the set of pins includes a spring configured to counteract a vertically oriented force provided by the cam card.
- 8. The system of claim 1, wherein the optical subsystem 20 comprises at least one unit including an excitation filter, an emission filter, a photodetector aligned with the emission filter, and a dichroic mirror configured to reflect light from the excitation filter toward the biological sample, and to transmit emitted light from the biological sample, through 25 the emission filter, and toward the photodetector.
- 9. The system of claim 1, wherein the magnet is arranged along a first side of the platform, and wherein the system further comprises a set of detection chamber heaters arranged along the first side of the platform.
- 10. The system of claim 9, wherein the cartridge heater is a plate heater directly coupled to the actuator and arranged along a second side of the platform opposing the first side, and wherein the first operation mode compresses the cartridge between the magnet and the detection chamber heaters at the first side, and the cartridge heater at the second side.
- 11. The system of claim 1, wherein at least one of the platform, the nozzle, the magnet, and the cartridge heater is coupled to a spring that provides a biasing force that 40 maintains proper alignment of the cartridge within the molecular diagnostic system in response to motion of the actuator.
- 12. The system of claim 1, wherein the actuator is a scissor jack actuator having an extended configuration in the 45 first operation mode, and a retracted configuration.
- 13. A method for processing a biological sample and separating a nucleic acid volume from the biological sample comprising:

receiving a cartridge between a cartridge platform of a 50 molecular diagnostic system, the cartridge platform having a magnet receiving slot, and an actuator opposing the cartridge platform, the actuator coupled to a

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nozzle of a liquid handling subsystem, an optical subsystem, and a cartridge heater; and

with vertical displacement of the actuator, simultaneously:

coupling the nozzle to a fluid port of the cartridge for receiving fluids for processing the biological sample from a liquid handling system upstream of the nozzle,

passing a magnet through the magnet receiving slot to interface with a first portion of the cartridge,

interfacing the optical subsystem with a second portion of the cartridge for receiving a processed derivative of the nucleic acid volume, and

compressing a third region of the cartridge between the cartridge heater and the cartridge platform.

- 14. The method of claim 13, further comprising separating the nucleic acid volume from the biological sample within the cartridge at the first portion of the cartridge upon passing the magnet through the magnet receiving slot, with vertical displacement of the actuator.
- 15. The method of claim 14, wherein separating the nucleic acid volume from the biological sample comprises occluding a pathway of the cartridge at a first set of occlusion positions to define a first truncated pathway, overlapping with the first portion of the cartridge, and processing the biological sample in a first processing phase within the first truncated pathway.
- 16. The method of claim 13, further comprising driving waste fluid derived from the biological sample into a waste chamber of the cartridge, upon applying a positive pressure through the nozzle and into the cartridge.
- 17. The method of claim 13, further comprising providing a spring coupled to the platform, the spring configured to counteract a vertical force provided by the actuator.
- 18. The method of claim 13, further comprising: with vertical displacement of the actuator, bringing the cartridge into an active configuration that disposes the cartridge proximal a set of pins interfacing with a cam card.
- 19. The method of claim 18, further including: displacing a subset of the set of pins in the active configuration of the cartridge, with translation of the cam card relative to the set of pins, to occlude a pathway of the cartridge; and separating the nucleic acid volume from the biological sample, within the pathway of the cartridge.
- 20. The method of claim 19, wherein displacing the subset of the set of pins comprises linearly displacing the cam card, comprising a set of hills and valleys, relative to the set of pins, wherein displacing the cam card affects an interface between the set of pins and the set of hills and valleys to transition at least one pin of the set of pins between a first position and a second position.

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